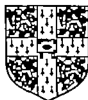
PROGRESS IN THE BIOLOGICAL SCIENCES IN RELATION TO DERMATOLOGY

EDITED BY
ARTHUR ROOK
M.D. M.R.C.P.
Consultant Dermatologist
United Cambridge Hospitals



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FOREWORD

Since the days of Harvey and Glisson, one of the characteristic features of British medicine has been the integration of medical science with clinical practice. In medicine today the well founded practical approach combined with human kindness is needed perhaps more than ever With emphasis on this background, it is essential for us to take part in, and utilize to the full, the advances in the scientific basis of medicine. In Cambridge, the development in the University of the School of Post-Graduate Medical Teaching and Clinical Research is rendered possible by the foundations laid as a result of the remarkable achievements in the fundamental sciences.

In post-graduate medical teaching, it is often useful to assess the present state of knowledge in certain fields of study It appeared that a good start could be made with Dermatology Largely as a result of the enthusiasm and energy of Dr Arthur Rook, a course on Progress in the biological sciences in relation to dermatology was organized. This book is a record of the course and has been prepared in the hope that it may be a contribution to medical learning I expect that this will be the first of a new series of Cambridge medical books.

J S MITCHELL

ADDENBROOKE'S HOSPITAL
CAMBRIDGE
10 December 1958

PREFACE

The lectures and discussions here presented follow very closely the programme of a course given in the Post-Graduate Medical School of the University of Cambridge from 22 to 29 September 1958. We are, however, fortunately able to include one of three lectures which Professor Mitchell was prevented by illness from delivering. The only other omission is Dr E. F. Gale's lecture on the bacterial cell: important commitments left him insufficient time to prepare his manuscript for publication.

The course did not attempt the impossible task of providing a comprehensive review of progress in the biological sciences in relation to dermatology. When it was planned many topics of great interest and importance to the dermatologist were deliberately excluded because they had formed the subjects of recent symposia or of readily accessible monographs. From the large range of topics worthy of inclusion those were favoured in which significant advances are taking place and which appeared to offer particular promise for future dermatological research.

The reports of the discussion have been edited and abridged. The wishes of the speakers themselves have been responsible for the omission of some interesting contributions, and lack of space has compelled the sacrifice of many more. The discussions, each under the chairmanship of a physician in clinical practice, were an important feature of the course. It is hoped that the reports as published provide a useful record of the lively exchange of ideas between basic scientists and clinicians which both found a valuable experience.

The arrangements for publication were not completed until long after the invitations to lecture were issued. It is a pleasure to thank the lecturers for their co-operation and tolerance.

It is a pleasure, too, to acknowledge my indebtedness to Professor J. S. Mitchell for the Foreword to this volume, to Dr Howard Whittle for devoting so many hours to the transcription of the tape-recordings, to Mr J. W. Woodcock, to Dr R. H. Champion, to the Librarian and Miss A. M. Jones of the Royal Society of Medicine, and to the staff of the Cambridge University Press.

ARTHUR ROOK

ADDENBROOKE'S HOSPITAL
CAMBRIDGE

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CONTRIBUTORS

ACKNER, BRIAN M.D., M.R.C.P., D.P.M.

Physician, Bethlem Royal and the Maudsley Hospitals. Lecturer in Psychological Medicine, Post-Graduate Medical School of London University

BOYD J D., M.D

Professor of Anatomy University of Cambridge.

BRADLEY P B., D.SC.

Department of Experimental Psychiatry University of Birmingham.

BRUCE, R. A. B.M., B.CH., M.R.C.P

Cambridge Chest Clinic.

BRUNET P C. J., D.PHIL.

Department of Zoology and Comparative Anatomy University of Oxford.

BURNS, M. B.SC.

Department of Pathology University of Cambridge.

CATER, D B., M.D F.R.C.S.

Department of Radiotherapeutics, University of Cambridge.

COOMBS, R. R. A., B.SC. PH.D M.R.C.V.S.

Department of Pathology University of Cambridge.

CULLEN J H M.B. B.CH.

Formerly Department of Medicine, University of Cambridge.

DAVIS, D RUSSELL, M.D., M.R.C.P., D.P.M.

Reader in Clinical Psychology University of Cambridge.

DIXON H. B F., PH.D

Department of Biochemistry University of Cambridge

DIXON KENDAL C., M.D

University Lecturer in Chemical Pathology Cambridge.

FOULDS, L., M.D

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London.

FOX, R. H., M.B. B.S.

Medical Research Council Laboratory Hampstead.

GALE, E. F SC.D., F.R.S.

Reader in Chemical Microbiology University of Cambridge.

GELL, P G H., M.B., B.CHIR.

Reader in Experimental Pathology Department of Experimental Pathology University of Birmingham.

GLEESON WHITE, M H., B.SC. M.B., B.S.
University Bacteriologist, Addenbrooke Hospital, Cambridge.

GLÜCKSMANN A. M.D
Strangeways Research Laboratory Cambridge.

GRESHAM, G A., M.D
University Lecturer in Pathology Cambridge

HARRIS, J IEUAN PH.D
Department of Biochemistry University of Cambridge.

HERBERTSON B M. M.D
Department of Pathology University of Cambridge.

HILTON S M., M.B., B.CHIR.
National Institute for Medical Research, Mill Hill, London, N.W 7

JARRETT A., M.B. CH.B., M.R.C.P
Dermatological Research Assistant, University College Hospital Medical School, London, W C.

MAGNUS, I A. M.B., B.CHIR., M.R.C.P
Institute of Dermatology British Post-graduate Medical Federation, London University

MITCHELL, J S C.B.E., F.R.S., M.D
Regius Professor of Physic, University of Cambridge.

MONRO P A. G., M.D., M.SC.
Department of Anatomy University of Cambridge.

PALMER, A. C. PH.D M.R.C.V.S.
Department of Veterinary Clinical Studies, University of Cambridge.

PARISH, W E., B.V.SC., M.R.C.V.S.
Department of Pathology University of Cambridge.

PATON W D M., F.R.S., D.M.
Professor of Pharmacology Royal College of Surgeons of England, London.

PEARSE, A. G EVERSON M.D., M.R.C.P
Reader in Histochemistry University of London. (Post-graduate Medical School.)

SCHACHTER, M M.D M.SC.
Department of Physiology University College London, W C.

SCOTT A., M.D.
Department of Dermatology St Bartholomew Hospital, London E.C. 1

SILVER, I A., M.R.C.V.S.
Department of Veterinary Anatomy University of Cambridge

SKINNER, M E.
Department of Pathology University of Cambridge.

SPECTOR, W. G., M.B., B.CHIR., M.R.C.P.
Department of Morbid Anatomy, University College Hospital Medical School, London.

TATCHELL, J. A. H., B.SC.
Department of Genetics, University of Cambridge.

WEDDELL, G., M.D., D.SC.
Reader in Human Anatomy, Department of Anatomy, University of Oxford.

WELLS, G. C., M.B., B.CHIR., F.R.C.P.
Physician, Skin Department, St Thomas's Hospital. Institute of Dermatology, British Post-graduate Medical Federation, London University.

WHITELEY, H. J., B.SC., M.D.
Institute of Pathology, Welsh National School of Medicine, Cardiff.

WHITLOCK, F. A., M.D., M.R.C.P., D.P.M.
Consultant Psychiatrist, Newcastle General Hospital. Formerly Consultant Dermatologist, West Cornwall Clinical Area.

WHITTLE, C. H., M.D., F.R.C.P.
Senior Physician, Skin Department, United Cambridge Hospitals. Associate Lecturer in Dermatology, University of Cambridge.

WOODHOUSE, D. L., PH.D., F.R.I.C.
Cancer Research Laboratories, Department of Pathology, University of Birmingham.

WRIGHT, G. H., M.D.
Department of Anatomy, University of Cambridge.

THE MELANOCYTE AND MELANOGENESIS

THE EMBRYOLOGY AND COMPARATIVE ANATOMY OF THE MELANOCYTE

By J D BOYD

I. ORIGIN OF MELANIN PRODUCING CELLS IN VERTEBRATES

One of the more remarkable of the many remarkable phenomena in vertebrate development is that provided by the behaviour of the so-called *neural crest*. This embryonic organ is an ectodermal derivative which becomes apparent during that period when the neural plate sinks in and separates from the overlying ectoderm to form the tubular primordium of the central nervous system. With this separation and the consequent fusion of the ectoderm from the two halves of the body in the middle line, the respective spheres of interest of neurologist and dermatologist would appear to have been nicely delimited by an embryological process.

But, as with other things, embryological processes are often not exactly as they seem! During, or indeed, even before, the separation of the neuroderm from the ectoderm a special group of cells can be identified in the transitional region between these two embryonic derivatives. It is now well established (for reviews see Hörstadius, 1950, and Boyd, 1955) that these intermediate cells constitute a special embryonic organ with most important functions in subsequent development. Although His (1868) gave the first description of the intermediate region, which he called the *Zwischenstrang* it is to Milnes Marshall (1878), who worked in the Zoology Department here in Cambridge under Balfour that we owe the name neural crest. During the eighty years since it was named, many embryologists have investigated, both descriptively and experimentally the neural crest and its derivatives in many vertebrate types. Even to summarize the results of all this work would require a course of lectures. For our present purpose, therefore, it must suffice dogmatically to state that the neural crest derivatives behave almost as a fourth germ layer thus they are known certainly to produce the dorsal root, and some of the cranial, ganglia, and the Schwann sheath cells of peripheral nerves. It is also extremely probable that they are the source of the sympathetic cells and the associated chromaffin cells of the body Moreover much elegant experimental work has demonstrated that the neural crest gives origin to a whole series of mesenchymal structures including the leptomeninges, much of the branchial cartilage, the odontoblasts, and, even, some membrane bone. Finally

and of more obvious relevance to the dermatologist, with the exception of those in the eyes,* the neural crest is the source of the pigment cells of the vertebrate body including all of those which produce melanin in the skin.

It was Borcea (1909) who from observations on the embryo of a Teleost fish, first suggested that the vertebrate pigment cells are derived from an ectodermal source in the neural crest region. In 1912 Weidenreich made a similar suggestion for the origin of these cells in amphibia. Meanwhile Harrison (1910), from observations on cultured frog spinal cord had predicted that the pigmented cells he found in his explants would prove to be of neural crest origin.

When the problem came to be investigated experimentally in Amphibian larvae (see Du Shane, 1943 1944 Rawles, 1947*a*, 1955 Willier 1948 Horstadius, 1950 for reviews of the literature) Harrison's prediction and Weidenreich's suggestion were strikingly vindicated. The evidence included (1) the total absence of pigment cells, in the appropriate region, following extirpation of the neural crest (neural folds) (2) production of pigment cells *in vitro* by explanted neural fold and (3) the appearance of pigment cells following grafting of neural fold material into the flank region of embryos of the same or a different species. Experiments on chick embryos unequivocally (the adverb is Dr Rawles') established a similar neural crest origin for the pigment cells in this vertebrate class. Of particular importance was the experimental demonstration that, when grafted in birds, the neural crest material invariably produces cells whose melanogenic activity in the host feathers is such that typical donor coloration and pattern result.

In mammals, as might be expected from the difficulties of experimental work on embryos of this vertebrate class, the evidence for the neural crest origin of the pigment cells is less weighty Nevertheless the work of Rawles (1947*b*) quite conclusively demonstrated that, in the mouse, the pigment cells are also of neural crest origin. This investigator grafted various portions of mouse embryos (of a homozygous black strain) to the coelom of White Leghorn chick embryos. The data on the results of these transplants showed clearly that only those tissues containing presumptive neural crest, histologically recognizable neural crest, or cells migrating from the neural crest can produce melanin-containing cells. Moreover skin and hairs which were normal in every respect save for the complete

* It should be noted that the pigment cells of the eyes may not, in fact, be an exception. Thus Conel (1942) has suggested that the neural primordia of the eyes may, in part at least, be homologous with the neural crest material associated with other levels of the developing central nervous system Rua (194) indicated that the retinal pigment arises in situ but that the pigment of the uveal tract is produced by cells of neural crest origin Further Bartelmez (1954) has suggested that the developing optic vesicles are, themselves, the source of neural crest mesenchyme (that is, ecto-mesenchyme) It can also be noted that the uveal tract corresponds to the leptomeninges

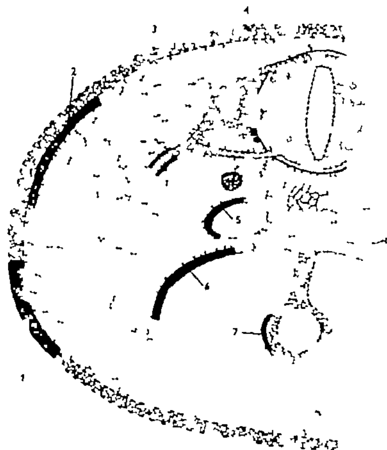
absence of pigment and pigment cells were regularly obtained in appropriate grafts whenever the neural crest had been excluded. As Dr Rawles writes The development of structurally normal white hairs from a potentially pigmented mouse is conclusive proof that the formation of melanin pigment in the ectoderm and its derivative, the hair is entirely dependent upon one particular type of branched pigment-forming cell—the melanophore—which originates in the neural crest.

II. DISTRIBUTION OF MELANIN PIGMENT IN VERTEBRATES GENERALLY

Such, in outline, is the embryological background to vertebrate pigmentation. But what is the distribution of pigmentation in the various tissues of adult vertebrates? Again, the relevant literature is enormous and here only a few of the more striking phenomena can be briefly considered. Since, at the commencement of their differentiation from the neural crest the melanin-producing cells are usually colourless, they cannot be identified during the early stages of their migration through the embryonic mesenchyme. Later when (and if!) they come to contain pigment the melanocytes* can readily be located. A most remarkable variation in their distribution in the different vertebrates can then be found (Text-fig 1). In the skin, for example, melanocytes may be found in the epidermis, or in the dermis, or in both of these tissues. They may be present universally in the skin or only in special parts of it. In the epidermis the pigment cells may be restricted to this layer in the narrow sense of the term, or they may be present in certain of its derivatives, such as the hairs or feathers. Or again, they may be present in both epidermis and its derivatives. Moreover in the epidermis, the melanin is not restricted to the cells, of neural crest origin, which produce it. The melanocytes here possess the ability to transfer the pigment they produce to the true epidermal cells (see

* There are many synonyms for the term melanocyte and real terminological problem is posed by the pigment cells I later stages of their development and in the mature condition (see Pl. 2, figs. 6 and 7) they possess number of ramifying cytoplasmic processes which led to Becker' (927) naming them *dendritic* cells. This term is descriptively suitable though there are, it should be noted, other cells in the body for which it would also be not inappropriate. *Melanocyte* is the term which is most usually used for them and which has the support of many important investigators. It has the disadvantage that although it is used for cells containing melanin there are many cells in different situations in differen vertebrates which correspond in origin and in morphology with the melanocytes but which, either permanently or intermittently are free from melanin Moreover the epithelial cells into which the pigment has been introduced do fall into the category of melanin-containing cells. Finally there is difficulty in finding name for the cells in the stage before they become melanogenic. *Melanoblast* is not really appropriate for such cells and the better terms *melanocytoblast* or *premelanocyte* are rather cumbrous In any case, such cells may be ancestral to melanocytes which will not (normally at any rate and in some instances probably never) produce melanin. Further discussion of terminology will be found in Gordon (953) Medawar (953) and in Billingham & Medawar (1953).

Billingham, 1949) or to the constituent cells of hairs (see Rawles, 1953) or of feathers (see Strong 1902) The process whereby the melanocytes transfer their melanin granules to the epithelial cells of the epidermis is not yet fully understood. It may be that the melanocytes actively transfer the



Text fig Schematic section through trunk region of vertebrate to show regions in which melanin pigment is commonly found. epidermis, 2, dermis 3 peri-neural sheath 4, meninges 5 peri-vascular adventitia 6 parietal coelomic wall 7 lateral coelomic wall

pigment to the passive epithelial cells by a kind of micro-inoculation It is possible however that the epithelial cells are active in the transfer by a phagocytotic, or some similar process. Whatever the mechanism of transfer it is a phenomenon unusual enough as Medawar (1953) has stated, to deserve the special name—cytocrine activity—which Masson (1948) has used for it. In the mesoderm I have never observed transference of melanin from normal melanocytes to adjacent non melanocytic cells. Such transference can be observed, however in melanotic tumours, and

occasionally melanin is found in macrophages and in reticulo-endothelial cells.

In addition to the pigment cells in skin, and ignoring those in the eye, there are, in the different vertebrate classes, many other regions containing active melanocytes. The variation is enormous and a complete list of the possible sites in which the melanocytes can be found would include nearly every tissue and organ. The commoner ones are indicated in Text-fig 1 they include (1) the meninges (2) regions along the course of peripheral nerves and of the sympathetic chain of ganglia (3) a perivascular situation in relation to the adventitious coat of the major blood vessels (4) the wall, usually the parietal but sometimes the whole, of the coelomic cavity or cavities. In addition melanocytes may be found in any mesodermal tissue. Thus in many lower vertebrates they are found in the interstices of muscle and in periosteum and perichondrium. Mucous membranes (for example, of the mouth or the lower urogenital tract) may also possess them. The variations in distribution of these melanocytes in the depths of the body are such as to defy any facile analysis attempts to explain their positions, either teleologically or in terms of survival value, leave one very puzzled indeed. Certainly in these deep situations the melanocytes cannot be concerned with biological reactions to extraneous radiant energy in, or near the wave-lengths of light, for such energy cannot reach most of them. Nor can they be explained in terms of protective or warning or sex-display coloration for it is only the anatomist who sees them.

III DISTRIBUTION OF MELANIN PIGMENT IN MAN

The neural crest of early human embryos is similar to that of other mammals (Bartelmez & Evans, 1926 Baxter & Boyd, 1939) The initial crest material is easy to identify in early somite embryos. As development proceeds, however apart from the cells contributing to the dorsal root ganglia, the elements arising from the neural crest lose themselves in the general embryonic mesenchyme and cannot be identified. Indeed, even in negroes (Zimmermann & Cornbleet, 1948 Zimmermann 1955), it is not until the

High energy short-wave radiation can, of course, reach the depths of the body It would seem extremely unlikely however from the apparently random distribution of melanocytes in the depths of the body of different vertebrates, that the cells are related biologically in any protective way to such radiation Indeed the greying of hair is known to be sensitive and lasting indicator of damage from ionizing radiations (Chase, 949 Chase & Rauch, 950). Chase & Post (956) have also shown that thin-down from cosmic ray heavy nucleus can cause the affected hair follicles of mice and guinea-pigs, exposed for several hours at high altitudes (about 00,000 ft.) and northern latitudes, to produce white hairs instead of coloured ones by irretrievably damaging the melanocytes. I know of no observations on the effects of radiation on the deeply situated normal melanocytes of the vertebrate body The cells of melanotic tumours are, however notoriously resistant to X-ray therapy

4·6 month of gestation, when the foetus has a C.R. length of well over 100 mm. that melanin appears in the melanocytes thus enabling these neural crest derivatives to be identified. However even in foetuses of white races, pre melanocytes can with the use of appropriate histological techniques, be identified at very much earlier stages. One such method depends upon the reduction of silver nitrate either by a pre-melanin or which is less likely by an enzyme concerned in melanogenesis. In my own material (Boyd, 1948*a* 1948*b* 1950*a*, *b* *c*) which includes a large number of silver impregnated human embryos, dendritic melanocytoblasts can be identified in the mesoderm and in the epidermis as early as the 40 mm. C.R. length stage. In pig embryos my material shows that they can readily be found distinctly earlier certainly from the 25 mm. stage (Pl. 3 fig 13) It is rare to find the argentaffin pre melanocytes in the general mesenchyme (Pl. 4, fig 20). Occasionally however they can be identified in the dermis, particularly in that of the skin of the back, and, a point of some interest, they are common in the connective tissue of the orbital cavities and in that related to the developing internal ear The foetal melanocytoblasts can readily be found in the epidermis (Pl. 1 figs. 1 and 4) and in the roots of hair follicles (Pl. 3 figs. 13–17). A region in which they are obtrusive is the epithelial plug of the developing external auditory meatus (Pl. 2, fig 8). Medawar (1953) and Billingham & Medawar (1953) have insisted on the expendable nature of the epidermal melanocytes, considering that they constitute, in post natal skin, a self reproducing system of which the older elements gradually pass up into the more superficial epidermal layers, where, eventually they are sloughed off with the epidermal cells of the stratum corneum. It may be that the presence of large numbers of dendritic melanocytoblasts in the epithelial meatal plug and in other regions of epidermal fusion (for example, the eyelids, Pl. 2, figs. 11 and 12, and the prepuce, Pl 1 fig 2) is due to the fact that these are regions where temporarily the surface layer cannot be sloughed off Thus the strongly argentaffin cell shown in Pl 2 fig 9 may be an effete embryonic dendritic melanoblast. Argentaffin dendritic cells are also present in large numbers in the buccal mucosa (Pl. 1 fig 5) and in the epithelium of the urethra (Pl. 1 fig 3), including that of the glands derived from it (Pl. 4 fig 21).

In my human foetuses I have not been able to identify melanocytes, or their argentaffin fore-runners, in the pia-arachnoid although in the adult such a distribution is not uncommon. In pig embryos, however pigment cells in the pia are often found. Indeed in some silver impregnated pig embryos, unfortunately of unknown race in my possession there is a rich distribution of dendritic argentaffin cells in the choroid plexus (Pl. 4, fig 18). In these same embryos dendritic-like glial cells can be identified (Pl. 4,



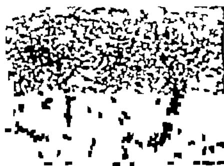
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PLATE 2



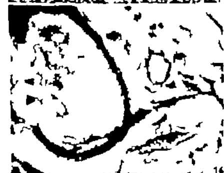
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For explanation see p. 4

fig 19) in the developing cerebellum just at the time when the ones in the choroid plexus appear This association may perhaps, be an indication that the neural crest is the source of these glial elements.

In every position in which I have found dendritic melanocytoblasts in white human foetuses the occurrence of melanin pigment has been recorded in post natal stages. Sometimes the pigment is present in otherwise normal individuals, for example, in the dermis of the back region (the Mongolian spot), the buccal mucosa, and in the pia-arachnoid. Such pigment would seem to be present more frequently in members of the negro or Mongolian races than in Caucasians. In other regions, as in the urethra and the vagina, the pigmentation may normally be present (though I, personally have not observed it in such situations in otherwise normal white individuals) but the presence of facultative melanocytes is revealed in Addison s disease and in pregnancy The hormonal alterations, probably an increase in the amount of ACTH or of intermedin, in such conditions also augment the pigmentation in the buccal mucosa and, of course, widely in the skin. The associated response of all the melanocytes of the body in such conditions and the reverse, the pallor shown by them generally in certain hypopituitary states, argue strongly in favour of their physiological unity There are also other conditions which demonstrate an affinity between the melanocytes of the skin and those of the depths of the body Thus those rare cases in which meningeal neoplastic melanosis occurs in association with naevi (Lecoutrier Ley Titeca & van Bogaert, 1939) constitute a striking example of the relationship of the cutaneous and the meningeal melanocytes. Further the occurrence of pigment deposition in conditions, such as neurofibromatosis, that primarily involve derivatives of the neural crest other than the melanocytes (for example, the chromaffin cells, the sympathetic cells and the Schwann cells) adds pathological evidence to support the strong embryological evidence for a special relationship between all the cells of the body that are of neural crest origin. Anyone concerned with problems of cutaneous pigmentation should keep this relationship in mind. Indeed I believe that, reciprocally embryology might be advanced by the study of the distribution of pigmentation in conditions such as cerebro-cutaneous melanosis. Detailed accounts of this distribution might give a hint as to the method of regional dispersal of melanocytoblasts in normal development. It is conceivable that some of those cases are due to a somatic mutation occurring in a cell derived from the neural crest. The final location in the body of the lineage of such a cell could help in explaining the normal pattern of dispersal of neural crest elements.

IV MIGRATION OF MELANOCYTES

That the melanocytes, in embryonic stages, can, and do migrate is abundantly clear We are, however largely ignorant of the factors controlling their migration. Weiss & Andres (1952) have provided evidence in favour of a selective association of embryonic pigment cells with specific body regions. These workers injected dissociated chick embryonic cells, including melanocytoblasts, into the blood stream of chick embryos. Instead of a random distribution of these embryonic pigment cells it was found that the resulting melanocytes proliferated and synthesized melanin specific for their own genotype only in those regions of the host in which, normally they would have developed pigment in the donor The melanocytes in the host, it is stated, were never found in unusual cell or tissue associations. Weiss (1947 1950), indeed, had already expressed the opinion that embryonic cells can only survive and achieve their developmental potencies in those locations that possess the specific conditions (chemical, physical and physiological) and the cell associations appropriate for them. According to this view embryonic cells of a particular strain can only become located in specific niches for which they are specially adapted. There is, however little evidence to indicate why the melanocytes came to settle down in the appropriate regions. It may be that those which find themselves in other areas either die or fail to propagate. Some of them may remain in a pigment less condition until an effective stimulus causes them to become melanogenic this may be the explanation of some of the atypically situated melanocytes referred to earlier Finally of course, it is possible that melanocytoblasts having found a particular region unsatisfactory continue to migrate until conditions are appropriate. Regions in which they never settle down will be those that are permanently colourless.

But while the evidence for migration of melanocytoblasts is completely convincing mature melanocytes appear for the most part to have lost the power to move Thus there is much evidence indicating that if the melanocytes in a pigmented region are destroyed, melanocytes from adjacent areas do not invade the region and consequently that region is lacking in pigment for the remainder of the animal's life. There are, however exceptions to this statement. Billingham & Medawar (1948 1950) have shown that pigmentation appears in the white epidermis of spotted guinea pigs when it is grafted adjacent to a pigmented area. These investigators suggested that white dendritic cells (non pigmentary melanocytes) in the basal layers of the grafted white epidermis became infectively transformed by contact with the normal melanocytes of the adjacent pigmented skin. The spread of pigment formation in such circumstances would not,

of course, be evidence for migration of fully differentiated melanocytes. Rawles (1955), however has argued strongly that all of the available data on pigmentation in mammalian skin grafts can be explained on the basis of the migration of melanocytes. She considers that, so far as recessively spotted rodents are concerned, white skin areas are melanocyte free. If such areas, following transplantation, became pigmented then, according to Rawles, the melanocytes responsible for this pigmentation must have migrated into them. The crucial point is the presence or absence of dendritic cells in the white skin areas. It is well established in fowls, and the same phenomenon appears to be true of mammals, that the presence of a normal complement of melanocytes in an area tends to block the entrance of other such cells. There seems, in fact, to be a relatively constant ratio between the number of melanocytes and of epithelial cells in birds (Willier 1948). Szabo (1955), however found a wide range of variation in the number of melanocytes between corresponding skin areas in different human individuals and in different parts of the skin of a single individual. In surface skin of face, forehead and ears he found 2000–4000 melanocytes per mm.^{2} in other body areas the number varied between 500 and 2000. Differential growth rates in different skin areas after the period of melanocytoblast migration may be the explanation of these variations in final melanocyte number It also seems that these numbers show a gradual decline during post natal development. In naturally hyperpigmented areas, such as the areola, the number of melanocytes per unit area was no greater than that in the surrounding skin. Szabo consequently concludes that it is qualitative changes in the melanocytes, rather than an increase in their number which are responsible for hyperpigmentation.

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EXPLANATION OF PLATES

PLATE

Fig. 1. Argentaffin melanocytoblasts in epidermis of cheek skin of 92 mm. white human foetus. × *circa* 450.

Fig. 2. Section through fusion of prepuce with glans penis in 150 mm. white human foetus (de Castro technique). The argentaffin cells in the glans (lower part of photograph) are still partly in the connective tissue. × *circa* 500.

Fig. 3. Argentaffin dendritic cell in basal layer of urethral epithelium of 92 mm. male white human foetus. × *circa* 450.

Fig. 4. Dendritic cell with argentaffin granules in epithelium of buccal mucosa of 92 mm. white human foetus. Processes of the cell extend through the basal layer towards the underlying mesenchyme. × *circa* 450.

Fig. 5. Argentaffin dendritic cells in epithelium of buccal mucosa of 50 mm. white human foetus. × *circa* 450.

PLATE

Fig. 6. Argentaffin dendritic cells of epithelial plug in external auditory meatus in 92 mm. white human foetus. × *circa* 200.

Fig. 7. Argentaffin dendritic cells of buccal mucosa in 92 mm. white human foetus. × *circa* 200.

Fig. 8. A row of argentaffin dendritic cells in meatal epithelial plug of 92 mm. white human foetus. × *circa* 20.

Fig. 9. *A deeply argentaffin cell in superficial part of meatal plug epithelium of* 92 mm. white human foetus. This is probably a premelanocyte that corresponds to Billingham & Medawar's superficial branched cell. × *circa* 450.

Fig. 10. Dendritic cell in basal layer of epithelium of buccal mucosa of 42 mm. white human foetus. Note the vacuolation in the cytoplasm of the branching cell. There is only slight evidence of silver reduction by the cell but its morphology is clearly not that of an epithelial cell. × *circa* 1000.

Fig. 11. Fused eyelids in 92 mm. white human foetus. An argentaffin dendritic cell can be seen extending across the line of epithelial fusion. × *circa* 120.

Fig. 12. Higher power view of dendritic cell shown in Fig. 11. As the fusion of the eyelids has occurred obviously this dendritic cell, the processes of which extend from the basal layer of the epithelium of one eyelid to that of the other, must have migrated in one or other direction.

PLATE 3

Fig. 3. Dendritic argentaffin cells in developing hair follicles of 39 mm. pig embryo. Such cells can also be seen in the epidermis.

Fig 4. Sections through hair follicles of eyebrow region in 92 mm. white human foetus. Note silver reduction by melanocytoblasts in papillary region of follicle and (by melanin or keratin) along the length f the hair shaft. × circa 20.

Fig 5 Root of hair follicle in 92 mm. white human foetus. Processes of melanocytoblasts are shown in the papillary region. Further up the shaft particles of reduced silver can be seen they are probably lying within dendrites of the melanocytoblasts. The shaft itself gives general reduction of the silver × circa 500.

Fig. 6 Root of hair in a 70 mm. white human foetus. A nerve fibre is approaching the follicle. Opposite the point where Schwann cell makes contact with the follicle an elongated dendritic cell is seen in the epithelium. Association of melanocytes with terminations of nerves is not uncommon. Possibly such cells migrate along nerves from the neural crest. circa 500.

Fig 17 Transverse sections though papillary region of hair follicle in the scalp of 15 mm. white human foetus. Note the heavy silver reduction by the dendritic cells and the absence of such reduction in the associated dermis. × circ 500.

PLATE 4

Fig. 8. Silver reduction by dendritic cells in choroid plexus f fourth ventricle of pig foetus. circa 20.

Fig 9. Silver reduction by glial cells with dendritic processes in periphery of developing cerebellum in same pig foetus. circa 350.

Fig. 10. Argentaffin dendritic cell in mesoderm of lower part f back in 92 mm. white human foetus. An elongated cell with long process showing some silver reduction is seen in the lower part of the field. circa 000.

Fig Argentaffin dendritic cells in epithelium of Cowper' gland of 92 mm. white human foetus. × circa 40.

MELANOGENESIS

By P. C. J. BRUNET

A DEFINITION OF MELANIN

Melanin is the term customarily used to denote certain stable pigments, usually but by no means always dark, commonly present in a wide range of animals and plants. It is unfortunate that the term has been used in many senses and has no precise and generally accepted meaning; it would be helpful if some agreement could be reached in settling the matter. Up to now the characteristic properties of melanin(s) have included (1) stability and relative insolubility, (2) derivation from phenolic precursors, or from one particular phenol, (3) by enzymic oxidation (and hydroxylation), (4) possessing redox properties, and (sometimes) (5) containing nitrogen.

Choice of an appropriate definition would seem to be largely a choice between a centripetal definition focused upon the best-known melanin, the pigment artificially derived by the *in vitro* incubation of tyrosine (or dopa) in the presence of tyrosinase, being essentially a polymeric structure based on 5,6-indole quinone (see p. 18). Much evidence points to the fact that this also is the essential structure of the natural melanin of mammalian choroid, retina, dark skin and hair, which therefore would be eligible to share the same definition as the artificial pigment. Other supposedly phenolic pigments, such as red hair pigment, which differ in a number of ways from black melanin, would not come within the definition.

On the other hand, an alternative would be a centrifugal definition, embracing the whole range of pigments which in the common use of the word have at one time or another been called melanins: these would include red hair and feather pigment, the pigment of freckled skin, the melanins of the insect eye, crustacean epidermis and cephalopod eye and skin, the last four of which are certainly derived from a phenolic precursor other than tyrosine (Butenandt, 1957). These would come under a general term melanin, meaning a group of pigments relatively insoluble in most or all organic solvents and water, more soluble in alkalis, derived from phenolic precursors by enzymic oxidation, being polymers formed by oxidative condensation, and showing redox colour change.

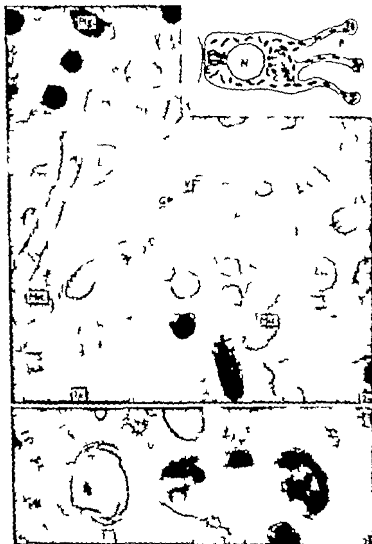
The general term would be qualified wherever possible with descriptive epithets, natural or synthetic melanin (Mason, 1948*b*) or presumptive melanin (Thomas, 1955), and, wherever possible, with a specific epithet, for instance when the substrate is known (Fitzpatrick & Lerner, 1954).

thus the human retinal melanin would be spoken of as a natural tyrosine melanin, and the insect eye pigment as natural 3-hydroxykynurenin melanin. This definition would have practical value, for it will take many years before the wide range of melanic pigments can be chemically defined, or their precursors identified, and in the meanwhile the pigments can graduate from the status of natural presumptive melanin to natural, let us say tyrosine melanin. In writing about a particular melanin, the full definition (with qualifying epithets) need only be used once, thereafter the word melanin being sufficient.

THE MELANOCYTE

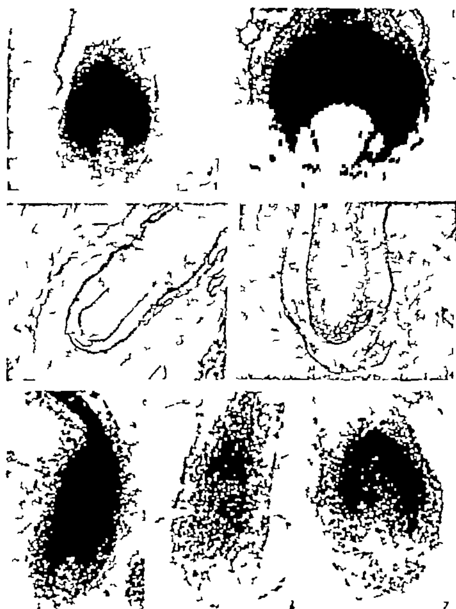
In all vertebrate animals melanin arises in particular dendritic cells known as melanocytes. The melanin may later be handed on from the dendrites to other cells (Billingham & Medawar 1948). Rawles (1947) has shown that melanoblasts of skin, and hair (or feathers) and of the uveal tract, migrate early in development from the neural crest. Melanocytes of the retina, however are part of the optic cup and thus represent part of the brain wall. Melanocytes have been described from other organs such as the Harderian gland and the nictitating membrane of mice (Markert & Silvers, 1956) and they may be even more widespread, occurring among the viscera of the silky fowl, and they are commonly found there in reptiles and amphibia. Pigment cells of human hair follicles (Pl. 1 fig 1) have recently been described from electron micrographs by Birbeck, Mercer & Barnicot (1956) these show the usual inclusions nucleus and mitochondria, and an infranuclear region of double membranes, such as are characteristically found in cells elaborating protein (in this case presumably the pigment granules). The pigment granules arise in vesicles, and have a lamellate form the early stages of the granule have the appearance of lamellate structures described by Chou & Meek (1958), which correspond with the Golgi body of the light microscopists. Danneel had in 1941 described the role of the Golgi system in pigment formation. Birbeck *et al.* explain the way in which portions of the dendrites of the melanocyte then stream off into the shaft of the hair They leave unsettled the question whether the nucleus plays a direct part in elaborating the pigment granule. A brief account of the ultra-structure of melanocytes in human skin is given in Barnicot & Birbeck (1958).

It is of extreme interest to find that differentiation of the melanoblast from neural crest tissue is directly dependent upon metabolic activities in which phenylalanine is a necessary substrate. Phenylalanine will even lead to the differentiation of anomalous melanoblasts (morphologically like

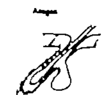
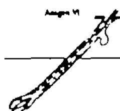
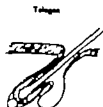


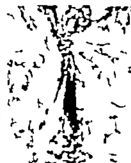
For explanation see p. 27

PLATE 2



For explanation see p. 27




For explanation see p. 27

normal ones) from ventral ectoderm (Wilde, 1955) Phenylalanine abnormally substituted on either the α or the β-carbon has not the differentiating ability of the natural amino-acid (Wilde, 1956)

THE PIGMENT GRANULE

The granules are the all-important structures with regard to melanogenesis they are the sites of pigment synthesis and of pigment deposition.

Despite the wide range of hair colour in man, it seems likely that the granules themselves will prove to have a very limited number of colours. Mice, which show an exceedingly wide variety of hair colours, have only three kinds of granule, black, brown and yellow (Russell, 1946). The number of colours in fowl feathers is also limited (Smyth, Porter & Bohren, 1951).

The size and shape of the granules are variable granules in human hair and skin are smaller (about 0·3 μ to 1·0 μ) than those in the retina (about 3 μ) Their shape and their deposition within the melanocyte can vary and thus affects the shade of colour of hair (Russell, 1946 Markert & Silvers, 1956).

Electron micrography leads to findings which are largely in agreement with the light microscopists work. The sub-spherical granules from mouse melanoma were isolated and examined whole by Mason, Kahler MacCardle & Dalton (1947), and lately Birbeck, Mercer & Barnicot (1956) have obtained micrographs of ultra-thin sections of granules. The mature granules are oval or rod-shaped and are very dense. They appear to arise as vesicles, in which lamellae are laid down. The vesicles eventually become completely filled with these lamellae (Pl. 1 fig 2). In albino hair the vesicles are present in the melanocytes, but there is no formation of lamellae. The greater ease with which red hair granules as compared with black are dissociated in alkali is reflected in the less dense appearance of the red granules.

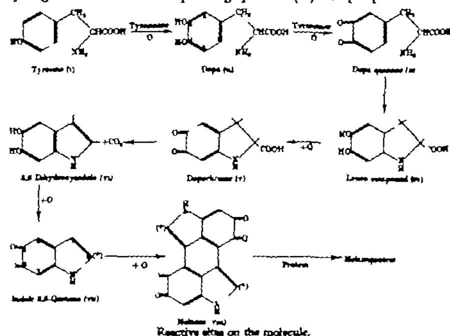
The pigment granules, certainly in vertebrate animals, are the cell components that contain the melanogenic enzyme (Riley *et al.* 1949). They also show anaerobic glycolytic activity and succinic and cytochrome oxidases* (Woods, DuBuy Burk & Hesselbach, 1949). Though they resemble mitochondria in their activities (DuBuy Woods, Burk & Lackey 1949), morphological evidence (Birbeck *et al.* 1956) does not bear out any such relationship.

* See Note added in Proof on p. 27

THE CHEMISTRY OF MELANOGENESIS

The classical work of Raper and his colleagues on the formation of artificial tyrosine melanin is now well known, and has been the subject of excellent reviews (Raper, 1928; Mason, 1955).

Essentially, melanogenesis is the change undergone by the colourless chromogen, tyrosine (i), to the insoluble melanic pigment (Text-fig. 1). Hydroxylation to dihydroxyphenylalanine (dopa) (ii) is followed by dehydrogenation to the corresponding quinone (iii). Dopa quinone is un-



Reactive sites on the molecule.

Text-fig. 1. Stages of melanogenesis (after Raper, Mason & Dalgleish)

stable and rearranges to form an indole compound, leucodopachrome (iv). Dehydrogenation to dopachrome (v) takes place, followed by decarboxylation and rearrangement to 5,6-dihydroxyindole (vi). It appeared that 5,6-dihydroxyindole (vi) was in some way converted into melanin (Raper, 1928). Beer, Clark, Khorana & Robertson (1948) synthesized 5,6-dihydroxyindole (vi), and found that it readily underwent oxidative polymerization to a melanin. This sequence was confirmed and extended by Mason (1948*a*), who also distinguished the red intermediate from hallachrome, showing that it was not identical with the red pigment from the annelid worm, *Halla*. He provided evidence that further oxidation of compound (vi) took place to the corresponding quinone (vii). Thereafter the mode of polymerization of compound (vii) was studied by Bu'Lock

& Harley Mason (1951*a*, *b*), by Beer Brown & Robertson (1951) and by Beer Broadhurst & Robertson (1954), admirably summarized in Mason (1955). Ideally the polymeric pigment forms through linkages of one monomer (vii) to another by way of positions 3 4 or 7 and sometimes 2.

The formation of even the synthetic pigment under controlled conditions is complicated by side-reactions the reactivity of the intermediate products makes possible their incorporation in the pigment polymer Under natural conditions, pigment formation is further complicated by the presence of reactive groupings on the proteins at the site of melanogenesis. The quinonoid pigment, or its precursors, will attach to side-chain or terminal amino-groups and to sulphydryl-groups. Natural melanins will almost inevitably owing to adjacent protein, prove to be composed of the quinonoid polymer intimately bound to protein. This indeed is indicated by the work of Greenstein (1948) who obtained, on digesting mouse melanoma protein with pancreatin, a residual black material with higher sulphur content than the native protein it would appear that the sulphur was contributed by the protein as sulphydryl groups to which the melanin polymer was attached. The linkages between quinones and amino-groups are very stable (Pryor 1940) and the presence of melanin might thus be expected to influence texture of hair indeed black hair and feathers are less subject to dissolution in alkali than are albino

THE ENZYME

An enzyme from plants that would oxidize tyrosine has been known since 1896 (Bertrand) Biedermann (1898) then demonstrated such a system in animals. Von Fürth & Schneider (1902) reported that the enzyme would act on tyrosine to yield melanin. As early as 1903 Gessard detected tyrosinase activity in horse melanoma and both Durham (1904) and Onslow (1915) carried out experiments on pigment formation in mammalian skin using tyrosine as chromogenic precursor

In 1917 however Bloch reported that human skin contained an enzyme that would oxidize only dopa (ii), but not tyrosine, to melanin. De Coulon (1920) then confirmed Gessard's claim that horse melanoma showed tyrosinase activity While Pugh (1933) detected tyrosinase activity in rabbit skin, both Russell (1939) and Ginsburg (1944) detected dopa oxidase but not tyrosinase activity in guinea pig skin. The situation regarding the melanogenic enzyme was confusing with no definite evidence to the contrary Bloch's (1917) claim that human skin showed only dopa oxidase activity remained acceptable. Again in 1942 melanomata (from mouse in this case) were shown to have tyrosinase (and dopa oxidase) activity by

Hogeboom & Adams (1942), and similar results were reported by Greenstein & Algire (1944). In 1944, Greenstein, Werne, Eschenbrenner and Leuthardt reported both tyrosinase and dopa oxidase activity from human melanomata.

The presence of both activities in melanomata (of mice) was again confirmed in 1949 by Lerner, Fitzpatrick, Calkins & Summerson. Furthermore they reached the conclusion that there was no necessity for postulating the presence of two enzymes for the two activities—a view that is now generally accepted (Dawson & Tarpley, 1951). The confusion had largely arisen because the enzyme's activity towards tyrosine was delayed by a long induction period unless small amounts of dopa were also present with tyrosine. Using Kubowitz's (1937, 1938) techniques, Lerner *et al.* (1950) showed that the activity of the enzyme was dependent on the presence of copper, and in 1951 the activity of the enzyme towards a range of substrates was investigated. By irradiating human skin prior to incubation with tyrosine Fitzpatrick, Becker, Lerner & Montgomery (1950) showed that the melanocytes could convert tyrosine to melanin. Further confirmation that hair and skin melanocytes in mammals other than man show tyrosinase activity has been provided by Foster using manometric methods (1951, 1952, 1956) and histochemically (1952).

The intractable nature of mammalian skin has so far prevented the isolation of pigment granules to allow critical manometric studies of their activity, and either pigment granules from melanomata, or whole skin have had to be used; recently, however, Miyamoto & Fitzpatrick (1957*b*), using fowl retina, have been able to study tyrosinase activity in granules isolated from normal tissue.

The extremely valuable histochemical method of Kukita & Fitzpatrick (1955) and Fitzpatrick & Kukita (1956), whereby tissue is incubated in ^{14}C labelled tyrosine, has made possible the exact localization of tyrosinase activity, and results have been obtained with human skin, hair follicles of man and other mammals (Fitzpatrick, Brunet & Kukita, 1958), and with fowl retina (Miyamoto & Fitzpatrick, 1957*b*).

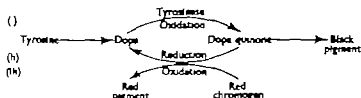
After a period of controversy it is now generally accepted that one enzyme, a copper protein, is responsible for tyrosinase and dopa oxidase activity in the skin, hair or feathers, and eyes of birds and mammals. Classical methods for separating proteins fail to fractionate the active protein. Whether the enzyme will show one or both activities or not depends on the chemical environment (see Fitzpatrick, Brunet & Kukita, 1958). Tyrosinase activity, for instance, will be suppressed if the copper of the enzyme is in the cupric form.

From a biological point of view this is interesting, for the means by

which enzyme activity may be suppressed in dormant melanocytes must lie in some such relationship between the enzyme and its cellular environments. A summary of the prevailing ideas about the melanogenic enzyme, and a plausible and stimulating speculation as to the role of copper in the composite activity of the enzyme has been given by Mason (1956).

THE SUBSTRATE

It is generally assumed that the natural substrate for melanogenesis is tyrosine (or perhaps dopa). With regard to natural black melanin this is probably true. Indeed the chromogenic stages of the natural reaction closely resemble these stages in the transformation of tyrosine, *in vitro* into pigment. Perhaps the strongest evidence comes from the finding that tyrosine administered to a patient suffering from phenylketonuria (where aromatic amino acid metabolism is upset) restores to normal the achromotrichia that accompanies the disease (Snyderman, Norton & Holt, 1955).



Text fig. 2. Diagram to show the suggested interaction between black and red pigment formation (i) Black pigment is formed in the absence of the red chromogen (ii) in the presence of red chromogen, dopa quinone is reduced (inhibiting the formation of black pigment) and (iii) simultaneous oxidation of the red chromogen to red pigment takes place.

Further evidence has been obtained by Brunet, Fitzpatrick & Kukita (1958) who have found that tyrosine, uniformly labelled with ^{14}C, or labelled in the side chain, when introduced into the eggs of fowls is rapidly incorporated into the feather germs of black chicks.

Red–yellow melanins may prove to have quite a different precursor If red hair follicles are incubated in tyrosine or dopa, the activity of the melanogenic enzyme is manifested by the formation of pigment, but this is *black* the golden red granules in human freckled skin behave similarly (Breathnach, 1958)

The presence of tyrosinase in red melanocytes would seem to implicate tyrosine somehow in melanogenesis, but there is no logical necessity for tyrosine to be the pigment precursor Red melanin is in many respects quite different from black its solubility properties are very different and many of the genes that affect its synthesis are without effect in an

otherwise identical but black, individual. Genes at the albino locus, that affect tyrosinase activity also affect the synthesis of red pigment.

It has been argued by Fitzpatrick *et al.* (1958) that the observed facts can be explained on a hypothesis that a second chromogen is involved in the formation of red pigment. The normal tyrosinase activity (Text fig. 2, i) takes place in red follicles. Meanwhile, the red chromogen is converted to red pigment by coupled oxidation (Text fig. 2, iii): as this oxidation of red chromogen takes place, dopa quinone is simultaneously reduced to dopa (Text-fig. 2, ii). Such a coupled system has been shown, *in vitro*, to convert amino-phenols to red pigments (Butenandt, Biekert & Linzen, 1956).

While red hair follicles, incubated with dopa, are found to lay down *black* pigment (presumably owing to lack of appropriate substrate), the follicles produce *red* pigment if incubated in a mixture of dopa and 3-hydroxykynurenin (Fitzpatrick *et al.* 1958).

MELANOGENESIS IN TIME AND SPACE

From what has been said, it is evident that a substantial amount is known about the chemical changes involved in melanogenesis, and about the enzyme that effects these. However for the highly organized body of a bird or mammal to carry out its ordained functions, these processes cannot take place at random: melanogenesis must be constrained to act at specific times in specific places. Such details have in some instances been investigated, but little is known about the mechanism of control.

It is more or less biologically axiomatic to say that melanogenesis is under genetic control. Total and local, albinism are inherited characters commonly found in animals. Studies on skin and hair of total albinos indicates that the lack of pigment is due to enzyme deficiency: it appears that the first stages of pigment-granule formation occur in albino human hair (Birbeck *et al.* 1956): but the results of many workers indicate no enzymic activity.

As might be expected there is no genetic condition leading to albinism through substrate (tyrosine) deficiency: for the side-effects upon, for instance, adrenal metabolism, would almost certainly make this condition lethal. The melanocytes, however, are effectively starved of substrate leading to achromotrichia, in the inherited disease, phenylketonuria, where an excessive amount of phenylalanine is present in the blood. Phenylalanine, differing from tyrosine with respect to one oxygen atom, inhibits tyrosinase activity by competing with tyrosine for the active sites on the enzyme (Miyamoto & Fitzpatrick, 1957*a*): oral administration of tyrosine will alleviate the lack of pigment in the hair.

A number of explanations of the mode of action of genes affecting hair colour has been given (Wright, 1941), and again recently in a most carefully reasoned paper Markert & Silvers (1956) have analysed the effect of the presence of mutant genes on the activity and embryonic differentiation of fifty different genotypes of mice from the point of view of morphogenesis of pigmented tissue this work is extremely important. The use of ^{14}C-labelled tyrosine has allowed quantitative determinations of enzymic activity in the hair follicles of a sample of genetically different mice and men (Pl. 2) (Brunet, Fitzpatrick & Kukita, 1958)

So long as the individual animal has the inherited capacity to synthesize pigment, it is likely that there will be variation in the activity of pigment synthesis at different stages in the life history Such variation has been shown very clearly in the retina of the fowl both by a histochemical method using ^{14}C-tyrosine, and manometrically There is no detectable tyrosinase activity in the retina of the adult fowl (or mouse, or human) but, during early development of the chick, activity rises to a peak at ten days, falling off rapidly until it has ceased by the fourteenth day (Miyamoto & Fitzpatrick, 1957*b*). It appears that there is one single cycle of activity The change in tyrosinase activity during hair growth in mice has been followed by Kukita (1957), using ^{14}C-tyrosine. He found that there is tyrosinase activity during anagen 3–6, but by the 24th day there is no further incorporation of tyrosine into the pigment (see Pl. 3). During telogen there is no activity but it is restored when replacement of the hair takes place. Ultimately decay of the enzyme takes place with the result that the hair produced is grey Kukita & Fitzpatrick (1955) have shown that grey human hair follicles have no active tyrosinase (compare Pl. 2, fig 1 with Pl. 2, fig. 4).

There is thus a growing body of observations on the behaviour of the melanocyte in the body in relation to genetic constitution and environment. Little is known about the actual *in vivo* control of melanocyte activity The melanogenic enzyme may be active or dormant dormancy may result from chronic failure due to inheritance, or nutritional deficiency or senescence, or there may be temporary cessation of activity The possible biochemical modes of control include competitive inhibition of the enzyme by substances related to tyrosine, or by agents such as sulphydryl-groups which block the copper atoms of the enzyme and the state of oxidation of the copper moiety will influence the activity of the enzyme towards tyrosine. There is inferential evidence that the substrate, tyrosine, may be held in a form unavailable to the melanocyte until liberated by a peptidase (Yasonobu, 1957).

Future steps toward understanding the biology of the melanocyte will

lie in discovering which of these controlling factors act to influence the biochemical process of melanogenesis at the various stages in the cell's life-history

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EXPLANATION OF PLATES

PLATE

Fig. Schematic diagram of melanocyte from human hair, showing the nucleus (N), granular double membranes (E) and the formative region (G) of melanin granules (M) which are mostly present in the dendritic process (P) of the cell.

Fig. 2a and b. Electron micrographs of portions of the cytoplasm of melanocyte.

Fig. 2a shows the formative ('Golgi') zone (G), where the melanin granules arise as small vesicles (V), then grow in size and develop internal lamellae (L), ultimately becoming pigmented (Pig). Mitochondria (Mit.) can also be seen.

Fig b shows developing melanin granules with greater and lesser amounts of pigment.

These figures were kindly provided by Dr M. S. C. Birbeck of th Chester Beatty Institute, Royal Cancer Hospital, London.

PLATE

Sections of hair follicles which, after incubation in ^{14}C-labelled tyrosine, have been mounted on radiographic plates Tyrosinase in the follicle cells converts th tyrosine into insoluble radioactive melanin. On development, black silver granules appear on the plates near the sites of melanin deposition.

Fig. 1. Radioautographs of black human hair The heavy deposit of silver granules indicates strong tyrosinase activity

Fig. 2. Radioautographs of red human hair The density of silver indicates strong tyrosinase activity but not as great as in black hair

Fig. 3 Radioautographs of albino human hair. The absence of silver granules indicates no enzyme activity

Fig. 4. Grey (aged) human hair The sparsity of silver indicates little or no enzyme activity

Figs 5 6 and 7 Radioautographs of mouse hair at anagen VI. Tyrosinase activity (indicated by the relative amounts f silver deposited) is determined by the hereditary factors that control hair colour The black follicle (Fig 5) shows intense activity the dilute black (Fig. 6) shows less activity and the yellow hair follicle (Fig. 7) intermediate activity

(From Fitzpatrick, Brunet & Kukita (958).)

PLA 3

Figs. 4 and 7 Diagrams of mouse hair follicles at anagen I anagen VI and telogen.

Figs. 2, 5 and 8. Sections of black mouse hair follicles corresponding to the diagrams stained with Ehrlich haematoxylin and eosin.

Figs. 3, 6 and 9. Radioautographs (see note referring to Plate) of black mouse hair follicles. Silver granules are absent from follicles at anagen I (Fig 3) and telogen (Fig 9), indicating no tyrosinase activity and no deposition of melanin. Silver granules at anagen VI (and also anagen II V which are not shown) indicate tyrosinase activity during these stages.

(From Fitzpatrick, Brunet & Kukita (958))

Note added in proof There is some doubt about the claim (p. 17) that melanin granules show succinic oxidase activity Results recently obtained by Dr Seiji and co-workers have demonstrated that in preparations of melanin granules from chick retina, from which mitochondria have been rigorously excluded, succinic oxidase activity is negligible. The activity is associated with the mitochondria only The purity of the fractions was checked by electron micrography

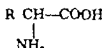
THE CHEMISTRY OF MELANOCYTE STIMULATING HORMONES

BY J IEUAN HARRIS

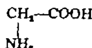
The presence of a melanocyte-stimulating substance in the *pars intermedia* of the mammalian pituitary was recognized over thirty years ago as a result of the investigations of Atwell (1919), Hogben & Winton (1922) Zondek & Kröhn (1932) as well as others on the ability of pituitary extracts to cause darkening of the skin in amphibia, particularly the frog. Darkening of the skin is believed to be due to the dispersion of melanin granules within the melanocytes under the influence of the hormone.

Most of the hormone substances which have been isolated from mammalian pituitaries have been found to be built up from amino acids which are joined together to form polypeptide and protein molecules. They range from comparatively simple polypeptides such as the posterior pituitary hormones oxytocin and vasopressin (du Vigneaud, 1954), corticotropin (ACTH) (for example, Bell 1954) which contains thirty-nine amino acids, to growth hormone, which contains from 200 to 400 amino acids depending upon the species from which it is isolated (cf Li, 1958)

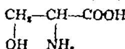
Amino acids have the general structure,



and differ from each other only by virtue of differences in the side-chain group R for example, when R = H we have



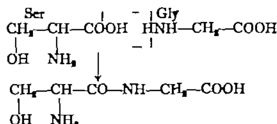
which is glycine (Gly) * when R = $CH_2(OH)$ we have



which is serine (Ser). In all approximately twenty different amino acids are found to occur in proteins of mammalian origin and they are linked together to form polypeptide chains by means of the peptide (CO—NH)

* The abbreviations for amino acids are in accordance with those proposed by Brand & Edsall (1947).

bond. For example, if we condense serine with glycine we get the dipeptide serylglycine with the elimination of a molecule of water



Serine which carries a free α NH_2 group is called the N terminal amino acid, while glycine which carries the free α-COOH group is called the C terminal amino acid.

ISOLATION OF MELANOCYTE-STIMULATING HORMONES

The isolation of a highly purified melanocyte-stimulating polypeptide from pig pituitary glands was first reported by Lerner & Lee (1955) and shortly afterwards the isolation of what appeared to be a different melanocyte stimulating substance was reported by three other groups of investigators (Porath, Roos, Landgrebe & Mitchell, 1955 Benfey & Purvis, 1955 Geschwind, Li & Barnafi, 1956). The substance isolated by Lerner & Lee (1955) was found to be a highly basic polypeptide with an iso-electric point of 11·0–11 5 whilst Porath *et al.* (1955) and Geschwind, Li & Barnafi (1956) reported iso-electric points of 5 2 and 5 8 respectively for the melanocyte-stimulating hormone (MSH) preparations which they had isolated. This apparent discrepancy was subsequently resolved in an elegant manner when Lee & Lerner (1956) were able to show that both forms of the hormone were in fact present in the same extracts of pig pituitaries. Consequently they proposed that the basic polypeptide which they had originally isolated be called α melanocyte-stimulating hormone (α MSH), and that the acidic polypeptide which had been isolated in the other laboratories be called β-melanocyte-stimulating hormone (β MSH). It should be mentioned that in the earlier literature MSH is frequently referred to as intermedin, melanophore-expanding hormone, melanin-dispersing hormone, and chromatophorotropic hormone.

This renewed interest in the isolation of pure MSH seems to have arisen as the result of speculations concerning its possible chemical and biological relationships to corticotrophin. This aspect of the problem will be discussed by Dr Dixon, and from what he will say it will be clear that pure corticotrophin must also be regarded as a melanocyte-stimulating hormone in its own right.

CHEMICAL STUDIES ON MELANOCYTE STIMULATING HORMONES

This section relates to the researches carried out in the Department of Biochemistry in Cambridge, in collaboration with Dr A. B Lerner Department of Medicine, Yale University and Mr P Roos, Institute of Biochemistry University of Uppsala, which have led to the elucidation of the chemical structures of both α and β-melanocyte-stimulating hormones from pig pituitary glands.

β Melanocyte stimulating hormone (β-MSH)

When a hormone has been isolated as a pure substance, that is, when a certain biological activity appears to be associated with a particular chemical substance, the work of the bio-organic chemist may be said to begin. The first thing he does is to break down the peptide hormone into its constituent amino acids by hydrolysis with hot concentrated hydrochloric acid, so that the exact amino acid composition of the molecule can be determined. When this procedure was applied to β-MSH it was found to consist of eighteen amino acid residues, in the following molecular proportions

Asp_2 Arg_1 Glu_2 Gly_2 His_1 Lys_2 Met_1 Phe_1 Pro_3 Ser_1 Tyr_1 Try_1

Having established the amino acid composition of β-MSH we were next confronted with the task of determining the order in which the eighteen amino acids are joined together to form the biologically active octadecapeptide structure of the hormone molecule This problem was investigated by Harris & Roos (1956 1959) using the methods which I shall outline below

N *and* C-*terminal groups*

As mentioned earlier two amino acids, namely the N and C terminal amino acids, occupy unique positions in a polypeptide chain and consequently provide convenient starting points for the initiation of structural studies. The N terminal amino acid in a polypeptide chain can be investigated by one of two methods the fluordinitrobenzene (FDNB) method introduced by Sanger (1945), and the phenylisothiocyanate (PITC) method, developed by Edman (1950). When a polypeptide is reacted with FDNB its N-terminal amino acid is converted into its dinitrophenyl (DNP) derivative. The DNP peptide is then hydrolysed with acid, the yellow-coloured DNP amino acid is separated from the mixture of free amino acids, and identified by chromatographic analysis on paper In Edman s method the N terminal amino acid is converted into its phenyl-

thiohydantoin derivative, which is then separated from the residual peptide and identified chromatographically

The C terminal amino acid, which carries a free α-COOH group can be investigated by a method which involves the use of the proteolytic enzyme, carboxypeptidase. This enzyme hydrolyses selectively the peptide bond which is adjacent to a free α-COOH group (that is, the bond by which the C-terminal amino acid is linked to the rest of the chain) the amino acid(s) released in this way may be separated from the residual peptide and identified by the standard methods of amino acid chromatography These methods of end-group analysis which I have outlined are described in detail by Fraenkel-Conrat, Harris & Levy (1955). When they were applied to β-MSH, aspartic acid (Asp) was found to occupy both the N and C terminal positions in the molecule.

Having identified the two terminal amino acids it became necessary to investigate those which occur in the interior of the chain. In general there are two main experimental approaches to the problem of determining amino acid sequences.

Partial hydrolysis

This method involves breaking a peptide chain into a number of smaller peptide fragments which can then be separated from each other and studied individually (see Sanger 1956). For this purpose specific proteolytic enzymes such as trypsin, chymotrypsin and pepsin are frequently employed. Thus trypsin is known to split peptide chains only at those bonds which involve the COOH groups of the basic amino acids, lysine (Lys) and arginine (Arg) while chymotrypsin has a marked affinity for bonds involving the carboxyl groups of the aromatic amino acids, phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Try) The presence of three basic amino acids and three aromatic amino acids, out of a total of eighteen amino acids, suggested that β-MSH would be a very suitable substrate for both trypsin and chymotrypsin and that separate hydrolysis by these two enzymes should give rise to relatively simple peptide fragments which would be suitable for further study

The enzymic digestions were accordingly carried out and the peptide fragments produced were separated from each other by means of chromatography and high-voltage electrophoresis on paper The amino acid composition of each peptide fragment was determined, and in most cases N and C-terminal amino acids were also investigated. Peptide fragments in which aspartic acid was the N-terminal amino acid were clearly derived from the N-terminal portion of the β-MSH molecule and conversely peptides with a C-terminal aspartic acid were derived from the C-terminal segment of the molecule. An examination of the amino acid composition

of each peptide revealed certain points of overlap which formed hinges around which a general over all structure could be based. These results, summarized in Table 1 enabled us to deduce a partial structure for β-MSH.

Table 1 *A partial structure for β MSH. Arrows show points of cleavage by trypsin* (T) *and chymotrypsin* (C)

Peptide	Deduced partial structure
Te / Ch (bracketed)	Asp† (Glu Gly Pro) Tyr
T	Asp† (Glu Gly Pro) Tyr Lys
Ch	(Glu His Lys Met) Phe
Te	(Glu, His, Lys Met Phe Arg)
T	(Glu His Met Phe) Arg
Ch	(Arg Try)
Ch	(Arg Gly Lys Pro, Ser Try) Asp*
T	(Gly Lys Pro, Ser Try) Asp
Ch	Gly* (Lys, Pro, Ser) Asp

Asp (Glu Gly Pro) Tyr C↓ Lys T↓ (Glu His Met) Phe* C↓ Arg T↓ Try C↓ (Gly Lys, Pro, Ser) Asp

* Amino acids released by carboxypeptidase. † N-terminal residues.

Step-wise degradation

Methods are available by which amino acids can be removed, one by one, both from the N terminal and C-terminal ends of a peptide chain. These methods, however are not of general application, and frequently break down when confronted with certain amino acids which act as structural barriers to further degradation.

Step-wise degradation from the amino end of the chain may be carried out by an extension of the Edman method for N terminal group analysis. By this procedure the amino acid carrying the free α NH_2 group is split off selectively as its phenylthiohydantoin derivative, which is then separated from the remainder of the peptide and identified by chromatographic analysis at the same time the α NH_2 group of the second amino acid in the chain (to which the N terminal amino acid was originally joined by a peptide bond) becomes free, so that by repeating the entire procedure this and other amino acids can be successively removed as phenylthiohydantoin derivatives Theoretically it should be possible to determine the entire amino acid sequence of a peptide in this way but in practice several as yet unresolved practical difficulties are encountered during its application.

When applied to β MSH however the phenylthiohydantoin method gave results which exceeded all expectation the hormone was submitted to ten successive applications of the method, and in this manner the amino acid sequence of the N terminal decapeptide was established to be Asp Glu Gly Pro Tyr Lys Met Glu His Phe. This result provided an

independent confirmation of the partial structure which had already been proposed for this part of the molecule, and also established the sequences in positions 2–4 and 7–9 in the molecule (see Table 1)

Step-wise degradation from the carboxyl end of a peptide chain can in some cases be accomplished by means of the carboxypeptidase procedure which I have already mentioned in connection with C-terminal group analysis. Following the release of the C-terminal amino acid the carboxyl group of the next amino acid to which it was joined in peptide linkage, becomes free, and on theoretical grounds this too should be susceptible to hydrolysis by the enzyme. In practice, however some amino acids do not conform to the specificity requirements of the enzyme and thus form structural barriers to further degradation. When this method was applied to β-MSH enzyme action ceased after the C terminal aspartic acid residue had been released from the chain.

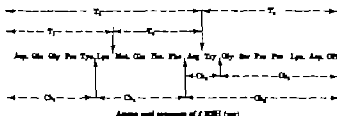


Fig. Amino acid sequence of β-melanocyte-stimulating hormone (pig, β MSH) arrows indicate sites of hydrolyses by trypsin (T), and chymotrypsin (Ch).

Wherever possible these step-wise methods of degradation were also applied to the peptide fragments which had been derived from β-MSH by digestion with trypsin and chymotrypsin. For example, in order to determine the sequence of amino acids in positions 13–17 (Table 1), peptide Ch_4, one of the products of chymotryptic digestion, was investigated by the phenylisothiocyanate procedure the phenylthiohydantoin derivatives of glycine, serine, and proline (twice) were successively released from Ch_4, and this result, together with the knowledge that it was the C-terminal peptide in the molecule, was sufficient to establish the sequence to be Gly Ser Pro Pro Lys Asp In this manner the last link in the chain was forged, and the complete structure of β melanocyte-stimulating hormone was established as shown in Fig 1 The same structure was proposed by Geschwind *et al* (1956) for the melanocyte-stimulating substance which they had isolated from pig pituitaries, showing that it was in fact identical with β-MSH.

The most interesting aspect of the structure proposed for β MSH is that it contains a sequence of seven amino acids (that is Met Glu His Phe.

Arg Try Gly positions 7–13 in the molecule) which also occurs in the corticotropin molecule. The significance of this finding will be discussed later

α Melanocyte-stimulating hormone (α MSH)

We next turned our attention to α MSH, the basic form of the hormone which had been isolated by Lerner & Lee (1955) The methods employed were similar to those which have been outlined above. Although α MSH did not contain either a free N terminal or C-terminal amino acid, the *complete structure of the hormone could be deduced from the structures* of the various peptide fragments which were derived from it by digestion with trypsin and chymotrypsin. As shown in Fig. 2, α MSH is the N acetyl derivative of a tridecapeptide amide which has the *same* amino acid sequence as the N terminal tridecapeptide segment of corticotropin (Harris & Lerner 1957 Harris, 1959)

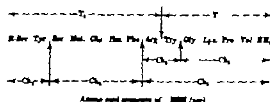


Fig 2. Amino acid sequence of α-melanocyte-stimulating hormone (pig MSH); arrows indicate sites of hydrolysis by trypsin (T), and chymotrypsin (C). $R = CH_3CO$.

STRUCTURAL AND ACTIVITY RELATIONSHIPS BETWEEN THE MELANOCYTE STIMULATING HORMONES AND CORTICOTROPIN

The amino acid sequences of the two melanocyte-stimulating hormones, together with the related N terminal portion of the corticotropin molecule, are illustrated in Fig 3 The heptapeptide, Met Glu His Phe Arg Try Gly is common to all three molecules, and but for the interchange of lysine and serine residues between positions 6 and 14, respectively in β MSH the common sequence would extend to eleven amino acids. Thus, although MSH and corticotropin are not identical as was at one time suggested by Johnsson & Högberg (1952) and Sulman (1952) it is clear that their chemical structures are very closely related. As Dr Dixon will mention pure corticotropin has been found to possess melanocyte-stimulating activity in addition to its primary adrenocorticotropic properties and a chemical basis for this dual activity has now been revealed. It emerges that a single chemical substance such as corticotropin can possess more than one biological activity and that these biological activities can be correlated with specific sequences of amino acids in the molecule. This result is

highly reminiscent of earlier findings concerning the chemical and biological interrelationships between the posterior pituitary hormones oxytocin and vasopressin (cf. du Vigneaud, 1954–5).

α and β MSH appear to have comparable melanocyte-stimulating potencies when assayed in the frog, and from a comparison of the amino acid sequences of the two hormones it emerges that the chemical structure which is essential for MSH activity appears in both cases to be found within a sequence of eleven amino acids (that is positions 2–12 in α MSH and positions 5–15 in β MSH) since the N terminal tetrapeptide and C-terminal tripeptide sequences in β-MSH can be replaced by the acetyl serine and valine amide moieties, respectively in α MSH.

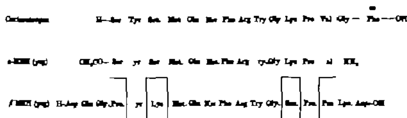
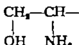


Fig. 3. Amino acid sequences of α and β-melanocyte-stimulating hormones, and the related N-terminal segment of corticotropin.

On the other hand, despite the fact that the entire amino acid sequence of α MSH is contained within its structure, the melanocyte-stimulating activity of corticotropin is from 100–200 times less than that of α MSH. As a possible explanation it could be envisaged that although corticotropin is potentially a highly active melanocyte-stimulating hormone, the additional structural features which are essential for its adrenocorticotropic properties may at the same time inhibit or mask its potential melanocyte-stimulating properties (cf. Harris, 1958).

The most obvious chemical difference between α MSH and corticotropin (apart from the actual length of the peptide chain) is to be found in the N-terminal serine residue of the two molecules in corticotropin the α NH_2 group is free, whereas in α MSH it is acetylated. (Incidentally this is the first time that an acetyl group has been shown to occur in a peptide or protein of mammalian origin.) Could it be that the



structure which has been shown to be essential for adrenocorticotropic activity (Bell, 1954 Dixon, 1956) is at the same time an inhibitor of

melanocyte-stimulating activity and does this explain why this structure is acetylated in α MSH? At present although there are no clear-cut answers to these questions, it is clear that corticotropin can be changed into a predominantly melanocyte-stimulating substance and that the N-terminal serine residue plays an important role in determining which of its two biological properties shall predominate.

In view of the striking chemical similarities which exist between corticotropin and the melanocyte-stimulating hormones it is tempting to put forward the suggestion that they may be synthesized by a common biosynthetic mechanism. Their location in different parts of the pituitary would seem at first sight to preclude any common mechanism of this kind, but the possibility that the two hormones are merely stored in the anterior and intermediate lobes respectively and that they may be synthesized elsewhere, possibly in the form of a common precursor molecule, cannot be completely overlooked. One might even ask whether MSH as such has any specific and independent function in the mammal. Could it not be a molecular appendix, an evolutionary relic whose function may have been superseded by the more sophisticated corticotropin molecule?

The chemical investigations which I have described have posed more questions than they have answered. The task of the chemist is nearing completion, that of the biochemist and endocrinologist is only just beginning. The many confusions and contradictions which at present litter the pages of scientific journals can only begin to be resolved if future biological and clinical studies are carried out on chemically defined and reproducible preparations of these hormones. Most, if not all, of the commercial preparations which are available at present are quite impure by chemical standards. This is particularly true of so-called "ACTH" preparations, and until the biologist and the clinical investigator *insists* on being supplied with highly purified preparations it is difficult to see how any real progress can be made towards elucidating the modes of action and physiological functions of pituitary hormones.

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MELANOCYTE STIMULATING HORMONES AND CORTICOTROPIN

By H. B F DIXON

The relationship between corticotropin and the melanocyte-stimulating hormones may be approached by a sketch of the history of our knowledge of the melanocyte-stimulating hormones. Atwell, of Buffalo New York, found in 1919 that tadpoles turned a silvery colour after their pituitary gland was removed, but returned to their normal black when placed in an extract made from the intermediate lobes of ox pituitary glands. Hogben & Winton (1922) at the Imperial College of Science shortly afterwards found that frogs placed in the light became a pale colour and that the injection of pituitary extracts darkened the skin, even when the nervous system was blocked by various drugs. Microscopic examination of the skin showed that the melanocytes varied in appearance from small round dots in the pale skin to highly branched star-shaped structures in the skin darkened by injections of what has since been called melanocyte-stimulating hormone or MSH. Much work has followed in purifying and isolating MSH and it has used this stimulation as the criterion for the activity

Naturally the change in the melanocyte was at first called expansion and it was thought that the cell put out processes like an amoeba. More refined microscopy has, however shown that the melanocyte of the frog is always star-shaped and that the apparent contraction is due to the streaming of the granules of melanin in towards the centre of the cell. This streaming leaves the processes empty and so invisible to the ordinary microscope.

Not only is there this rapid effect, but preparations which possess this MSH activity also showed, in the cases where they have been tested, the property of increasing the amount of melanin in the amphibian skin (Frieden & Bozer 1951 Karkun & Mukerji, 1953). Whereas the dispersion of melanin granules occurs in about half-an hour the amount of melanin gradually increases over some weeks of administration of the hormone.

Administration of MSH-rich preparations to man also leads to an increased pigmentation (Lerner Shizume & Bunding, 1954) but it does not seem to be known if this effect follows slowly after a rapid granule dispersion into the cell processes as it does in the frog What has been most studied is the effect of *mammalian* MSH on *amphibian* skin, and the mechanism of human pigmentation deserves more work.

The idea that the darkening of the skin in Addison's disease might be due to excessive MSH activity was confirmed in Sweden by Johnsson & Högberg (1952) when they found that there was a high level of MSH activity in the blood of patients with Addison's disease. This raised the question of corticotropin, otherwise known as ACTH the hormone that stimulates the adrenals to produce cortisol. Since the level in the blood of corticotropin was also high, Johnsson & Högberg suggested that MSH and corticotropin might be identical. Sulman (1952*a*) made the same suggestion and pointed out that all corticotropin preparations possessed high MSH activity

This suggestion of identity did overlook the fact that the intermediate lobe of the pituitary gland had always been found to be the richest source of MSH and the anterior lobe of corticotropin. Several workers showed that corticotropin activity could be destroyed without affecting the MSH activity of a preparation by various chemical treatments, but though this showed that the activities were due to distinct chemical structures, it was still possible that the two distinct structures might be possessed by the same molecule, so that the two hormones could be identical. But then Reinhardt, Geschwind, Porath & Li (1952) in California obtained a clear separation of most of the corticotropic activity of a preparation from most of its MSH activity and so concluded that the hormones were two different substances. This separation has since been confirmed by many others. Johnsson & Högberg (1953) withdrew their suggestion of identity while Sulman (1952) modified his to say that MSH is one factor of the ACTH complex I am not certain what this means, but perhaps it implies that the molecules of corticotropin and MSH are secreted together or even combined with each other Though they do seem to be secreted together under some circumstances, we must remember that there is an immense difference between pituitary anterior and intermediate lobes in their relative amounts of the two hormones (Landgrebe & Morris, 1955).

The clearness of separation that was obtained, and the isolation by several groups of workers of apparently pure MSH or in fact two chemically different MSH's called α MSH and β-MSH both devoid of corticotropic activity generated some scorn of the idea of any relationship at all between the two substances. It was merely conceded that both were concentrated together in a number of fractionation procedures due to some similarity of chemical structure. But although workers on MSH got rid of corticotropic activity from their substance, those who isolated corticotropin found that it retained some MSH activity Finally some of these workers (Winter Brink & Folkers, 1953 Bell, 1954) suggested that some MSH activity was an intrinsic property of the corticotropin molecule.

Bell and his colleagues (1954) in the American Cyanamid Company in Connecticut were impressed that in their isolation of corticotropin, once they had removed the main melanocyte-stimulating impurity the ratio of MSH activity to corticotropic activity remained constant whatever steps they took to fractionate the product. So they suggested that this residual activity was an intrinsic property of the corticotropin molecules.

The residual MSH activity in the corticotropin was less than 1 per cent that of MSH itself or in other words, to produce a given degree of darkening of the frog skin required over 100 times more corticotropin than MSH. This meant that this residual activity of corticotropin, if not intrinsic, would only need 1 per cent of contaminating MSH to account for it, and as this small amount could not be detected chemically further evidence was desirable to confirm Bell's suggestion that the activity was not due to impurity

The actual doses required are interestingly small. A pale frog is turned extremely dark by the injection of 1 μg of corticotropin or about 8 mμg of MSH. These are not particularly small doses in comparison with the normal action of corticotropin about 10 mμg corticotropin injected into a rat which itself may be ten times the weight of a frog will give a maximal stimulation of the adrenal cortex judged by its immediate effect. The size of the effective doses fit into a series of amplifications In man $\mu\mu$g of substances may be released at nerve endings, and these may act on the hypothalamus to cause release of mμg of hypothalamic substance which in turn acts on the pituitary to induce the release of μg of corticotropin. At each stage there is an amplification factor of a thousand. The series continues as the μg of corticotropin stimulate the production of mg of steroids which in turn affect the metabolism of g of fat, carbohydrate and such-like metabolites.

To try to get more evidence on whether corticotropin did have intrinsic MSH activity one of the reactions of corticotropin was studied and the product examined by chromatography Dixon & Stack Dunne (1955) had already used chromatography for the isolation of corticotropin. In this method a vertical column is packed with an absorbent powder and liquid is allowed to percolate slowly down through the column of the powder The sample to be separated is dissolved in a small volume of the liquid and put on to the top of the column. When it has flowed down into the powder the flow of the plain liquid is continued.

The separation works as follows any substances that were in the sample and that are strongly absorbed by the powder will remain on the top of the column and will not appear in the liquid that flows out at the bottom. Any substances that are not absorbed at all will move down through the

column at the same speed as the liquid and will emerge as an unretarded band. But substances which are reversibly absorbed to an intermediate extent will move down the column more slowly than the liquid and will form separated bands. The substance in one of these bands is at any moment in equilibrium between the powder and the liquid. The more strongly it is absorbed by the powder the greater is the proportion of the time that each of its molecules is in the stationary powder and not in the moving liquid, and so the slower band of this substance moves down the column. So the

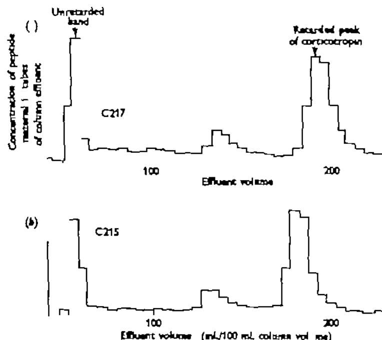


Fig (a) Chromatographic pattern obtained with concentrate of corticotropin (b) Chromatographic pattern obtained with peroxide-inactivated concentrate of corticotropin (Dixon & Stack Dunne, 953).

substances in the sample may be separated according to their affinities for the powder the substances with the higher affinities forming the slower bands.

If the liquid is collected as it emerges from the bottom of the column in a series of test tubes, the substances that have been separated on the column according to their relative affinities for the powder may be separately collected. The patterns shown in Fig 1 can be obtained when the amount of substance in each test tube is plotted against the volume of the liquid in the tubes as they are filled from the bottom of the column. First there is the unretarded band of the substances that are not appreciably held back,

and then peaks of more strongly adsorbed substances. The only difficulty in applying chromatography to purifying a new substance is in finding a suitable pair of adsorbent and liquid which will give the partial and reversible adsorption of the substance that is required for it to form a retarded peak.

Fig 1*a* shows the isolation of the main form of corticotropin from a concentrate prepared from pig pituitary glands. Most of the MSH activity emerges in the unretarded peak which is a mixture of substances, and there is the residual MSH activity in the corticotropin peak about which we wish to know whether it is intrinsic or not. If it were due to contamination it might be because the molecules of corticotropin formed a complex with MSH which held some of it back on the column from the unretarded position where it would otherwise emerge.

We were able to approach this problem of whether the MSH activity was intrinsic or not by applying what was known of the reaction of hydrogen peroxide with corticotropin. Dixon & Stack Dunne had noticed that after treatment with hydrogen peroxide, which was known to inactivate corticotropin, the peak emerged slightly earlier on chromatography as you may see by comparing Fig 1*b* of the chromatography of peroxide inactivated material with Fig 1*a* After this we used a powder of smaller particle size and obtained a separation, as shown in Fig 2, of native corticotropin separated from peroxide-inactivated material

The other datum about the peroxide reaction was that Dedman, Farmer & Morris (1955) at the London Hospital had discovered how to reactivate peroxide-treated material, which many other workers had been trying to do. They heated the material in the presence of a thiol compound to about 80 C for about 24 hr We were able to confirm this reactivation and to show (Dixon, 1955) that the reactivation was accompanied by restoration of the original chromatographic properties. Fig 3*c* shows a chromatogram of the native corticotropin. A sample was treated with peroxide and part of it was chromatographed (Fig 3*a*). It showed the earlier emergence of the peak. Part of this same sample was heated with a thiol and it has moved back to its original position (Fig 3*b*).

This was all known about the reaction of corticotropin with peroxide before any study was made of what happened to the MSH activity in the process. In fact the MSH activity of both corticotropin and MSH was lost on treatment with peroxide, *and* regained on heating with the thiol. But when peroxide-treated corticotropin was *partially* regenerated by heating with a thiol, chromatography gave separation of two peaks. The faster one was inactive in both MSH and ACTH tests, while the slower was active in both (Dixon 1956*a*).

Far the simplest interpretation of this is that both activities are intrinsic

to the same molecule, so that regeneration of the original molecules explains both the regaining of the original position on the chromatograms and also the recovery of both activities. The possibility that the peak owes either

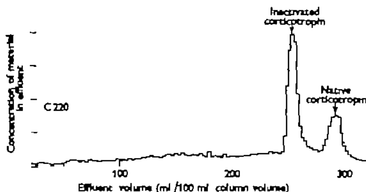


Fig. 2. Separation of native and peroxide-treated corticotropin.

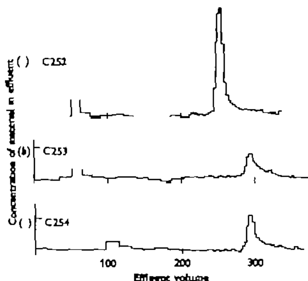


Fig. 3. ( ) Chromatogram f corticotropin after peroxide treatment. (b) Chromatogram of corticotropin after peroxide treatment followed by heating with thiol compound. (c) Chromatogram of untreated corticotropin (Dixon 955)

activity to contamination of its main component with a trace of impurity is rendered much less likely because not only does the peak show a change of chemical properties when the activity is changed, but also because only one of the two peaks is associated with the activity A complex might be

expected to be formed with both if the peak were merely active through holding back some MSH from the unretarded position. So the evidence that the MSH activity was intrinsic was strengthened.

I obtained other confirmation when I treated corticotropin with periodate (Dixon, 1956*b*). This specifically attacked one group in the corticotropin molecule and the product could be isolated by chromatography. It had lost its corticotropic activity, but not only had it retained MSH activity during this further isolation in a new form, but its MSH activity was increased. Although still much less active than α- or β-MSH, it had evidently been made more like them in some way, as Dr Harris's adjoining paper mentions when he deals with the common part of the structures of MSH's and corticotropin (Harris & Roos, 1956; Harris & Lerner, 1957).

The demonstration that corticotropin does possess MSH activity and the discovery of the features of structure and chemical behaviour that are common to both hormones, do not clear up all the outstanding problems of the relations between MSH and corticotropin. The known facts do not explain, for example, why the blood of patients with Addison's disease should be rich in MSH as well as in corticotropin. Nor why cortisone or cortisol treatment lowers the blood level of both hormones (Shizume & Lerner, 1954) while they affect the pituitary level of corticotropin much more than that of MSH (Forgács, 1956; Ivy & Albert, 1957).

The high MSH activity of corticotropin preparations, including all that are widely available for clinical use at present, has been simply explained by a study of the chemistry as due to gross contamination with MSH. The somewhat subtler problem of common activity was solved also by a study of the chemistry. But in the remaining problems on, for instance, the rate of secretion, the control of secretion and the levels of hormones in the blood and in the gland, the amounts of hormones available are in milli-micrograms, a million times or so less than the milligrams normally required for chemical work. One example of how far we are from being able to apply our chemical knowledge is that α- and β-MSH have been clearly distinguished chemically for some years, but of the relative amounts secreted and the relative localizations in the pituitary gland nothing is known. It does not seem that our chemical techniques are anything like sensitive enough to be well poised at the moment for further attack on the biological relations of these hormones.

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DISCUSSION

Chairman DR T B FITZPATRICK (Harvard)

FITZPATRICK. It is appropriate that this conference has begun with a discussion of melanogenesis, for although many questions are yet unanswered, perhaps more is known about melanin formation than any metabolic process in the skin. Not too many years ago a discussion of the phenolases in the fungus *Russula nigricans* would have appeared useless to the clinical dermatologist. As we have heard this morning, the metabolic pathway of tyrosine to melanin was worked out by Raper using plant and insect tyrosinases, and now this pathway has been shown to exist in mammals. With the rapid strides in melanin research in the past twenty years we are now able to state with reasonable certainty that human melanin pigment is formed by the action of tyrosinase attached to melanin granules approximately 0·2 μ in diameter which are suspended in the cytoplasm of certain specialized cells called melanocytes. This family of cells, the *melanocyte system*, includes the melanocytes in the skin, hair bulb pia-arachnoid, uveal tract and retinal pigment epithelium and all these cells are derived from the neural crest and outer layer of the optic cup (retinal pigment epithelium).

The amount of melanin formation in the melanocyte is delicately controlled and varies considerably in different physiologic states. The biochemical regulatory mechanisms of melanin formation are not understood and are being actively investigated at this time. For example, a pituitary hormone, melanocyte-stimulating hormone (MSH), appears to affect either the movement of melanin granules, the amount of melanin formation, or both. As Dr Harris has stated, we are not sure whether in man the melanocyte-stimulating hormone may be a metabolic appendix or a factor in melanogenesis. Other known biochemical regulatory mechanisms such as the inhibitory action of phenylalanine and sulphydryl groups affect melanin formation *in vitro* but their role in melanin formation *in vivo* is not yet clear

Even with our present knowledge, the clinician can better visualize the possible mechanisms involved in the large number of disorders of melanin pigmentation in man and has at his disposal specific histochemical methods for the detection of tyrosinase, for quantitative estimation of the number of melanocytes in the skin, and techniques for estimation of the amount of melanocyte-stimulating hormone in the blood and urine. For example, in albinism it has been shown that the mechanism of decreased melanin

formation is the absence of the enzyme tyrosinase pigmentless melanocytes and even pigmentless melanin granules are present, but the gene which serves as a template for tyrosinase synthesis is absent or abnormal. In Addison's disease, the increased melanin pigmentation is probably related to the high levels of blood MSH released from the pituitary in abnormal amounts because the diseased adrenal is not supplying adequate amounts of hydrocortisone to inhibit the pituitary secretion of MSH (Lerner Shizume & Bunding, 1954) Many further examples of the mechanisms of pigmentary disorders will be forthcoming as a result of the collaboration of the basic scientist and the clinical dermatologist.

DR J COHEN (*Birmingham*) *I would like to ask Professor* Boyd whether he considers that argentophil dendritic cells are present in all mammals in such areas as he has mentioned, in some mammals producing pigment in all these areas and in other mammals producing pigment in fewer of them. In the rat the hair is frequently pigmented, pigmentation may be found in the peritoneum and in the meninges but not in the epidermis or around peripheral nerves. Does *Professor Boyd consider that potential pigment* cells are present around these nerves and in the epidermis in this animal?

PROFESSOR J D BOYD (Cambridge) That is a very good question indeed. By and large I think the answer is yes one does, however find dendritic cells which are not melanogenic. This raises a question of terminology *To talk about a non-melanogenic melanocyte is more than a little awkward* but I think it does answer your question to say that, in many regions, there are facultatively non-melanogenic melanocytes. I do not know if there are such cells along nerves in rats. My own material has been largely human, pig and rabbit, and some shrew I do not know the state of affairs in the rat. To answer your question *fully* would require a detailed survey of suitably stained material in this mammal.

DR G C WELLS (London) If you go back to an early stage of cell division as, for example, in frog spawn, there is much pigment production. Is this melanogenesis a usual property of primitive cells which is later lost, or should we regard melanogenesis as a specialized function of certain cells?

DR P C J BRUNET (*Oxford*). *It is difficult to give an exact answer* The embryo is endowed from the parent with pigment which I think simply peters out. It just remains there until it gets dispersed and then, I think, the new melanin starts developing in melanocytes.

BOYD That answer is certainly correct. *But I would like to protest against calling the ovum a primitive cell* it is the most specialized of all cells!

DR C D CALNAN (London). May I ask Professor Boyd a question about the melanoblasts in the epidermis have these cells actually migrated into

that position or are they perhaps induced by an organizer which modifies the basal cells? Is the melanoblast mobile?

BOYD The answer is quite clearly that all the melanoblasts, melanocytes and melanocytoblasts, or whatever name one wants to use, in the epidermis come from the neural crest. Cultures from embryonic skin at a stage before the pigmentogenic forerunners have reached the dermal level show quite conclusively that the epidermal cells themselves are not melanogenic. There is no evidence that melanocytes can induce other cells to become melanogenic. Indeed all the evidence supports strongly the view that the melanocytes constitute a self perpetuating special strain of cells.

FITZPATRICK Most human and animal biologists have accepted the terminology of pigment cells adopted at the Third Conference on the Biology of Normal and Atypical Pigment Cell Growth, held in 1951

Melanoblast An embryonic cell potentially capable of producing melanin.

Melanocyte. A *mature melanin-producing and melanin-containing cell.*

Macrophage A cell containing phagocytized melanin.

Melanophore A pigment effector cell in lower animals.

There is fairly general agreement also on the neural crest origin of melanocytes, although some still maintain without any experimental support that the epidermal melanocytes are derived from the basal cells.

The identification of amelanic melanocytes is fraught with difficulties and I question the validity of the silver stains in the identification of melanocytes until a study of the type that Silvers (1957) reported on gold stain is carried out. Silvers found gold-impregnated cells in tissues without melanocytes, and thus his work appears to refute the commonly accepted notion that the gold-impregnation technique can be used for the identification of amelanic melanocytes. The electron microscope appears to be the most precise method of identifying melanocytes whether pigmented or amelanic, and considerable new information of the fine structure of melanocytes has been obtained by Barnicot & Birbeck (1958).

CALNAN May I ask Dr Brunet about the status of copper? There is a view that when one uses psoralens to treat vitiligo the addition of copper sulphate sometimes makes it more effective. Could he perhaps tell us what is the best form in which to give copper?

BRUNET I think it would be best to refer to Mason (1956) who adequately copes with the subject of copper It is known, though, that in the oxidized form it will catalyse oxidation of dopa, but you have to reduce it first to get it going on tyrosine. As regards the use of copper Dr Fitzpatrick and one or two others who have used copper could say more about this.

FITZPATRICK The potentiation of methoxsalen by the simultaneous

administration of copper salts has not been established by careful clinical trials and thus we are not certain that it is a fact. It has even been stated that the action of the various furocoumarins has been due to the content of copper as a contaminant. There is no copper in the preparations that we have been using in the U S.A. (meloxine, oxsoralen). About five years ago we administered 15 mg of copper sulphate daily to patients receiving methoxsalen there was not any acceleration of the rate or increase in the degree of repigmentation in those patients that received copper in addition to methoxsalen.

WELLS. Does not the pigmentation of patients with Addison's disease fade under treatment with DOCA? Does DOCA inhibit MSH?

FITZPATRICK. Although DOCA restores the metabolic abnormalities in patients with adrenal insufficiency the increased pigmentation is not restored to normal with this therapy (Thorn, Dorrance & Day 1942). Cortisone, hydrocortisone and 9-α fluorohydrocortisone, however will usually reverse the pigmentation in adrenal insufficiency presumably by inhibiting the pituitary secretion of melanocyte-stimulating hormone (MSH). Perhaps Dr Main would like to comment on this, also?

DR R. A. MAIN (Dundee) We have noticed some interesting cases of Addisonian and bilateral adrenalectomized patients on maintenance doses of steroids, who instead of remaining relatively normal in colour or losing their pigmentation as you might expect, have become more pigmented.

FITZPATRICK. By following the degree of pigmentation with the Hardy reflectance spectrophotometer during treatment of Addison's disease with cortisone, it has been shown that the melanin hyperpigmentation decreases with cortisone therapy (Hall, McCracken & Thorn, 1953).

DR G WEDDELL (Oxford). There does seem to be some kind of functional relationship between melanocytes and cutaneous nerve fibres. For instance, skin infected with myco-bacteria leprae is almost invariably hyperpigmented and there is good evidence that this disease is primarily an affection of the sensory cutaneous nerves. On the other hand, the only indisputable facts of which I am aware have just been presented by Professor Boyd, namely that there is a very close anatomical relationship between melanocytes and nerve fibres from the earliest stages of development through into adult life. I would like to ask Professor Boyd whether the density of innervation is greatest in zones of skin in which the melanocytes are most numerous.

BOYD I have no evidence that suggests a richer innervation to heavily pigmented areas. Masson (1948), however and other workers, have associated pigment-spots with nerve-endings.

FITZPATRICK. Dr Harris would you like to comment?

DR J T HARRIS (Cambridge) It should perhaps be emphasized that

the relative melanocyte-stimulating potencies of MSH and ACTH have been determined by assay of the mammalian hormones in the frog It is possible, however that the chemical structures which we have determined may have been designed to survive and to perform specific functions in a particular host environment, and that the relative potencies which we ascribe to MSH and ACTH, respectively may not apply in the mammalian systems from which they are isolated.

For example, α MSH which is composed of only thirteen amino acids may be more susceptible to enzymic inactivation than the larger ACTH molecule, consisting of thirty-nine amino acids consequently in the mammal, ACTH could be a more effective melanocyte-stimulating hormone than either α MSH or β MSH. In a similar manner peptide fragments of mammalian MSH which are found to exhibit some melanocyte-stimulating activity *in vitro* assay in isolated frog skin, might not have any detectable activity if they could be assayed under the appropriate physiological conditions.

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JS INNERVATION

THE STRUCTURE OF THE SKIN IN RELATION TO ITS INNERVATION

By G WEDDELL

It is common knowledge that the skin reflects changes in our physical and mental well being and it has been demonstrated, by plastic surgeons among others, that skin is not a static tissue either in form or function. Yet, until recently our knowledge of the structure of the skin was almost entirely limited to a series of words anatomizing pictures of thin sections under the microscope, taken either from preserved or recently dead bodies.

This important organ, which incidentally accounts for about 16 per cent of the body weight, is still analysed in most books in dreary descriptive terms, only occasionally enlivened by the names of famous biologists of past generations, such as Huxley and Henle, whose names are attached to hair sheaths which could gain little of importance as a result. This must be one of the factors which has effectively deterred most dermatologists from taking up any form of anatomical research on the skin, for by so doing they would add yet more descriptive terms to an already over rich and function ally meaningless vocabulary

To most of those who did venture into the histological wilderness the reward was meagre and I shall not easily forget my own first excursion. What, I was asked by a student, is the structural difference between skin from the arm of a wizened old man and skin from the arm of a well nourished and healthy young girl? I admitted my ignorance but undertook to find out, but the answer was not easily forthcoming A labour of elephantine proportions was spent on digesting the literature and all that emerged was a minutely proportioned mouse. In effect, histologically speaking the difference between the two was virtually nil. That this would be the answer should have been obvious, for preservatives and fixatives in common use cannot be expected to give pictures showing, among other things, the comparative state of turgor between one specimen and another

It is the ability to recognise in the skin a dynamic framework within which change is constantly occurring, which has recently led to such a hopeful increase of our knowledge of the structure of the skin. It is more especially the experimental work of Medawar (1953) and such investigators as Rothman (1954), Shelley & Arthur (1958), Fitzpatrick & Lerner (1950) and Montagna (1954) which has revolutionized our outlook in this field. They have taught us that mammalian skin is best thought of in dynamic

terms as a compound holocrine gland (in which is embedded a series of eccrine glands) its secretions being keratin melanin sebum, sweat and possibly heparin and histamine (mast cells). It is still agreed that a useful purpose is served by subdividing the skin into two parts for descriptive purposes, that is into dermis and epidermis The epidermis, however is now seen as a binary system of two cell lineages, the keratinocytes and the melanocytes The dermis is seen as the supporting fibrous tissue framework for the epidermis and the remaining glands, and contains blood vessels, lymphatics and nerve fibres. In addition, the dermis in some way induces and regulates the differentiation of epidermal cells and, making use of this fact, it has been possible to prove that the various forms taken by epidermal cells are not rigidly determined, they are pluri potential cells biased in a given direction. This discovery has of course far reaching implications for the dermatologist and plastic surgeon.

Viewed from this angle the skin is an ever-changing structure, liable to both cyclical and continuous growth changes, yet it remains at all times a communication barrier with the outside world. It is in this role as a communication barrier that the skin behaves as an integrated organ and unity of function is exhibited by both the epidermis and dermis. This is seen particularly well in any form of stress such as exposure to high environmental temperatures.

Turning now to the sensory innervation of the skin, the subject of this symposium, it is my purpose to show that the static theory of the permanence of its structure has here, too, outlived its usefulness, and the rest of this paper will be devoted to an elaboration of this point.

It is a fact that despite the wear and tear of everyday life, the sensory innervation of the skin is capable of giving us day by-day an adequate picture of our surroundings and moreover we are perfectly capable of recognizing by touch the shape and consistency of objects before and after say rock-climbing in which the hands suffer a considerable amount of damage It has also been established that no diminution of sensory acuity can be detected with advancing age as the skin loses its bloom of youth and becomes progressively more wrinkled and scarred. These observations alone suggest that the sensory cutaneous nerves must either be constructed of tissues which can withstand greater stress and strain than the tissues among which they lie, or they must be so arranged that the kind of information which they signal is not dependent on the integrity of each and every sensory termination which is present.

The evidence available suggests that some of the sensory cutaneous nerves do indeed suffer damage in the course of everyday life and are just as labile as tissues such as blood capillaries. This evidence is necessarily

indirect, for it is not possible, as yet, to watch the behaviour of living nerve fibres in the intact unoperated skin. Fortunately however it is possible to demonstrate living nerve fibres and their terminals for short periods in the human conjunctiva and to see whether they undergo any morphological changes, either with the passage of time or as the result of being subjected to methylene blue staining I shall now give a brief account of our findings.

Until the present time there has been considerable controversy as to the presence in the conjunctiva of specialized sensory end bulbs Some authors maintain that Krause end bulbs are not only present in the human conjunctiva but distributed in such a way that they can only be considered as the specific endings subserving cold sensibility others maintain that in the majority of animals it is not possible to demonstrate any end-bulbs in the conjunctiva. The vituperative nature of the outbursts in the literature between the most eminent of authors on the subject of the presence or absence of end-bulbs is unusual. In retrospect, the explanation for this excessively high level of emotional tension should have been obvious end-bulbs may or may not be present in the normal conjunctiva. Proof that this is so however was not easily established for it depended upon a quantitative survey of conjunctivae from numerous eyes taken post mortem, coupled with an examination of living methylene-blue stained conjunctivae in which the nerve fibres and their terminals were selectively outlined.

It was my colleague Dr Oppenheimer (Oppenheimer Palmer & Weddell, 1958) who discovered the trick of vitally staining nerves selectively in this situation. It is a very simple but important element in a technique which had previously been tried out but which gave very inconsistent results. It is simply necessary to ensure that the eye is anaesthetized with cocaine before the bathing with methylene blue starts, for the dye stimulates the nerves before paralysing them and so gives rise to great pain, tears and excessive vasodilatation unless this is done.

In both specimens and subjects which we examined we found that the great majority of the nerve fibres entering the conjunctiva end as diffuse arborizations of fine filaments in relation to blood vessels or to the epithelium of the conjunctiva and cornea these are commonly known as free nerve endings. Compact nerve endings (or end-bulbs) were extremely rare in the conjunctiva of the common laboratory animals but they occur in variable numbers and are irregularly distributed in man They are of different types including end-bulbs of the kind described by Krause and there are a number of features, such as their shape, their vulnerability to methylene blue, etc., which suggest that they represent stages in a cycle of growth and decay in certain peripheral nerve fibres rather than specialized sense

organs. To elaborate on this point, we noticed that the nerves serving end-bulbs were both larger in diameter and more irregular in outline than neighbouring fibres in the same bundle which, when traced to their destination, were found to end in widespread arborizations of fine filaments (free nerve endings). Moreover we were impressed with the wide range and often bizarre appearance of the end bulbs The over all picture presented resembled very closely indeed that described by Cajal in the proximal stumps of nerve trunks a few days after they had been divided, for the terminations ranged in form from simple growth cones through rather more elaborate end-clubs to nervous spools and the apparatus of Perroncito It is noteworthy that no distinctive capsular cells can be defined surrounding the neural elements of such end-bulbs only enlarged Schwann and epineurial sheath nuclei are seen In other words, the innervation constitutes the whole of the organ. Perhaps the most persuasive finding of all in favour of the lability theory is the presence in a few specimens of stem axons with widespread arborizations of fine filaments (a free nerve ending) springing from one healthy looking daughter branch and an end bulb surmounting the other branch which was itself thickened and irregular

Although, before staining with methylene blue, we made no elaborate tests which might have enabled us to compare the sensory capacity of the various conjunctivae in a refined manner we did carry out simple standardized tests which were of some comparative value using warm and cool copper rods and nylon threads of varying thicknesses. The results were unequivocal in that the four primary sensory modalities, touch, warmth, cold and pain could be evoked in every case (whether the conjunctivae subsequently proved to contain either a large number of end bulbs or no end bulbs whatever) at thresholds which did not vary significantly

Finally although we have not examined a very large number of conjunctivae vitally stained with methylene blue, there is, in our series, a significant correlation between the presence of numerous end bulbs and the age of the subject. Few if any were found in children or young subjects, particularly those with bright clear whites to their eyes.

If we are correct in our interpretation of these findings then in a turbulent tissue such as the skin evidence of similar cycles of growth and decay might well be expected With our findings in the conjunctiva to guide us we searched for and have now found similar cycles in the skin, although we have never seen as many *end-bulbs* per unit area in apparently normal skin as we have seen in the conjunctivae of apparently healthy subjects.

At this point I must digress for a moment to emphasize how much more difficult it is to demonstrate neural elements in the skin in an undistorted

form as compared with the conjunctiva. In the past we managed to develop techniques to cope with skin from specific zones in laboratory animals but it was quite another thing to develop a technique for staining nerve fibres in human skin of varying thickness and structure from a variety of regions. However more of my colleagues came to the rescue and Dr Graeme Schofield (1958) and Dr Elisabeth Palmer (Weddell, Jamison & Palmer 1958) have at last developed a suitable technique for this purpose. It is a silver impregnation method (modified Bielschowsky-Gros) applied to very thick sections (200 µ) from skin biopsies removed with a punch 4 mm. in diameter between 5 and 10 min. after infiltration of the skin with 0·5 per cent procaine containing hyaluronidase.

Clearly we were not likely to obtain the evidence we sought by taking a few casual biopsies from wives, friends, colleagues and neighbours, even supposing that we could manage to persuade them to assist us in this way but since we are at present engaged in an analysis of the sensory and neurohistological changes occurring in the skin in leprosy we were able to make use of the control specimens from this material.

Careful examination of a large number of specimens of skin from various regions of the body made it clear that there are no end bulbs in hairy skin borne at the ends of nerve fibres which are both normal in diameter and in contour In apparently healthy and undamaged skin, however we have come across a significant number of degenerating and regenerating axons but only on very rare occasions are they surmounted by end bulbs similar to the smallest of those seen in the conjunctiva.

In connection with the problem of the lability of cutaneous neural elements we were fortunate to be in a position to study skin infected with leprosy for leprosy is a disease which primarily infects the sensory cutaneous nerves. In our studies of this infected skin two things stood out clearly in the first place, it was highly significant that, despite histological evidence of the destruction at random of up to at least a quarter of the nerve fibres of all sizes in a given piece of skin, we found that it had not been possible to demonstrate by any means known to us any loss of sensory acuity although in the most severe cases before biopsy the patient had reported that the affected skin did not feel quite right when he stroked it.

In the second place, in hairy skin with a certain form of leprosy in which the Schwann and epineural sheaths were enlarged and mycobacteria leprae were found in the cytoplasm of the cellular elements of these sheaths but not elsewhere, a number of end-bulbs of all sizes quite similar to those seen in the conjunctiva were found. End bulbs were less common in hairy skin in which the Schwann and epineural sheath cells were less active despite the fact that mycobacteria leprae were both more numerous

and more widely disseminated in the skin. There seems to be no doubt that in leprosy the presence of end-bulbs in hairy skin is correlated with the amount of physical obstruction offered by the products of the disease process to regenerating nerve fibres.

Confirmation of this notion is provided by the presence of end-bulbs in hairy skin taken from patients with post herpetic neuralgia in which nerve regeneration had occurred but in which the sensory acuity was diminished, and in the skin over painful scars covering amputation stumps. Mycosis fungoides is another disease in which Dr Kreutzberg (1957), a pupil of a German colleague of mine, Professor Emmi Hagen, has found numerous end-bulbs in hairy skin, although changes in the affected skin are said not to be a noteworthy feature in these cases.

Observations of this kind, then, suggest that it is not unreasonable to conclude that sensory cutaneous nerves are labile in nature, that they can and do undergo cyclic variations in contour in numbers and less commonly in the form of their endings, to adapt themselves to the wear and tear of everyday life as well as to trauma and to disease processes affecting the skin. Any theory of the mechanism of cutaneous sensibility is thus more likely than not to depend upon the recognition of coded patterns than upon a point to point projection of specific modalities.

There is much additional evidence of an experimental nature which bears out this conclusion and I should like to refer briefly to the anatomy of the innervation of the hairs of the rabbit ear for I believe that the innervation of the hairs in human skin is based upon a similar principle, although more work will have to be done before this contention can actually be proved (Weddell, Taylor & Williams, 1955).

The innervation of the hairs of the rabbit ear is highly complex. In the first place the number of hairs exceeds the number of sensory neurons serving them on the average there are 100,000 hairs served by myelinated axons from 6000 neurons. Moreover degeneration and electrophysiological experiments make it clear that every hair is served by axons from at least two neurons, some by as many as twenty an average hair is served by six neurons. The situation is further complicated by the fact that the areas covered by different neurons varies, but it is usually large often amounting to one half of the total area of the ear The amount of overlap between the areas served by different neurons is thus very great.

Our studies also make it clear that the size of the axons serving each neuron varies over a narrow but significant range, certainly sufficient to ensure that there is a wide range of conduction velocities so that stimulation of a single hair will produce a shower of activity at the cord which is patterned in time as well as in space. All these observations taken together forced us

to the conclusion that any theory of animal behaviour based on the notion that there are peripheral units, hairs, having exclusive lines connecting them with the spinal cord are unlikely to be correct, for stimulation of a hair in the rabbit ear must lead to a space time pattern of activity in which a number of neurons (shared by other hairs) are involved. If man resembles the rabbit in this respect—and there is every reason to believe that he does—then the ability to localize a hair must depend upon the ability to differentiate between complex space time patterns projected on to the spinal cord it cannot depend upon the recognition of a particular nerve fibre which exclusively innervates a particular hair

The advantage of such an arrangement in the skin is obvious it means that a proportion of nerves can fall out at random without altering the over-all pattern signalled when a piece of skin is stimulated. In other words, a measure of protection remains, as far as hair sensation goes, until most of the sensory cutaneous nerves have been destroyed. Indeed, such an arrangement can be regarded as a communication system having reliability and also economy for it employs the minimum number of components necessary to signal the maximum amount of information.

The fact that hairs do not have private lines of communication with the central nervous system but share them with other hairs, and that the neural arrangements are such that the information they signal reaches the central nervous system in the form of a coded pattern of impulses, does not mean that the whole problem of the mechanism of cutaneous sensibility is solved, but it does mean that the theories put forward in text-books will have to be revised at least in respect of hair touch.

There are some recent observations, however which do take us a step further For instance, it has been shown that hair bulb endings do not discharge in reponse to heat exchange, that is either to warmth or to cold moreover they discharge their information rapidly (by myelinated nerve fibres) into the dorsal columns of the spinal cord. In man, therefore, they must be concerned primarily with touch sensibility

Meissner corpuscles in the human finger pads are the counterpart of hairs elsewhere in the skin and must be presumed to have comparable functions and central connections. They are certainly supplied by nerves which are arranged in much the same way in the skin (Cauna, 1954) and it is not without interest that, in skin which is normally hairy but in which the hairs have fallen out, the hair bulb endings come to look very much like Meissner corpuscles. Moreover the sensory acuity in such zones of skin is of a high order Both hairs and Meissner corpuscles can therefore be regarded as specialized touch receptors.

The other primary sensory modes are served by the free nerve endings.

Whether warmth, cold and pain each are served by a physiologically specific set of such endings is still uncertain although recent studies of a physiological nature (Douglas & Ritchie, 1957) suggest that these endings have only a restricted degree of specificity

From the anatomical point of view these endings cannot be differentiated from one another but they overlap and interweave extensively and terminate at different levels in the skin. Unlike hairs they are served by nerves having a wide range of fibre diameters but they are arranged in the skin in a similar way to those serving hairs. It also seems certain that the free nerve endings feed their information to the antero-lateral columns of the spinal cord (spinothalamic tracts). Thus, although there is still a lot which remains unknown it is certain that isolated modality-specific endings having private lines of communication with the cerebral cortex do not exist. Information from the skin must reach the central nervous system in the form of a coded space/time pattern of action potentials.

From this I think it follows that although we are still quite ignorant as to the mechanism of paraesthesiae such as itch it would seem logical in the first place to regard them as resulting from a disturbance in the regular pattern of activity reaching the brain, rather than resulting from the activation of a particular group of nerve fibres and/or their cutaneous terminals which are normally silent. This is not to say that in a given case paraesthesiae may not result from the over activity of certain specific terminals but rather to emphasize that it is the particular pattern of activity which reaches the brain which is ultimately responsible for the sensation which is perceived, and that this pattern may be generated anywhere between the skin and the brain itself

To sum up then, the skin is labile both in structure and function and the sensory nerves which supply it are also labile and in this way adapt to changes in their surroundings. The skin is also an organ of exteroception and its sensory nerve supply is arranged in a complicated way which appears to be related to the fact that the information it signals to the central nervous system is relatively unaffected by the cyclical changes which both tissues undergo Clearly these important facts, among a host of others, must be taken into account when erecting an hypothesis concerning the mechanism of cutaneous sensibility

I am well aware that I have omitted many points of importance and interest as regards the structure of the skin in relation to its innervation, but the scope of this subject is a very wide one and it would take too long to discuss all the relevant points. Those who will be following me will certainly supply some of these deficiencies and help to give you a clearer picture of this complex subject which is, in my opinion of considerable importance to the practising dermatologist.

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THE EFFECTOR INNERVATION OF THE SKIN

By P A. G MONRO

There has been a vast amount of research on the vasomotor nerves to the hands and feet, fingers and toes, but until recently there has been relatively little work done on the nerves to blood vessels in other areas of the skin of the body We know much more about the pathways to the skin concerned with sweating The determination of the sweating patterns to be observed on patients after operations for sympathectomy has enabled us to investigate these sudomotor pathways much more thoroughly

I would like to consider some sweating patterns observed on patients who have undergone different types of sympathectomy In this short paper it is not feasible to give you a complete account of all the patterns to be observed on patients after all types of sympathectomy Only representative patterns can be considered here but a full account of more than fifty cases has been published elsewhere (Monro 1959). These patients suffered from hypertension, Raynaud's disease or other peripheral vascular disease, and include a case of brachial plexus injury

All the patients were in the care of Mr D W C. Northfield of the Neurolosurgical Department of the London Hospital.

The method used to demonstrate sweating activity was that of measurement of the electrical resistance of the skin after inducing the patient to sweat freely by heating in a hot air cabinet. In general the patterns are not entirely symmetrical but the front of the thigh down to the medial malleolus of the ankle always retains sweating activity as does the lower abdomen. Other patients who have had sympathectomy at these levels show similar patterns and it is evident that they are based on the patterns of the dermatomes as described by Foerster in 1933

Foerster showed that there is considerable overlap between adjacent dermatomes on the thorax, for instance, this overlap extends over the immediately adjoining intercostal spaces. Foerster's observations, in this respect, are very *similar to those previously made by Sherrington* on the macaque monkey (1893 and 1898). In particular I wish to consider the first lumbar dermatome extending over the groin and upper and outer thigh and buttock the second lumbar dermatome including almost all the front of the thigh down to the level of the knee and also a separate portion over the buttock and the third lumbar dermatome including the

inner side of the thigh and down on the calf to just above the medial malleolus with another separate part over the buttock.

The patterns of sweating observed on patients were photographed and then transferred to standard diagrams for convenience in comparing the patterns on other patients. Fig 1 shows the pattern observed on a patient (E.L.), who underwent bilateral sympathectomy T 4–L 3 and was examined three weeks and eight weeks respectively after the time of operation. It is apparent that the pattern is essentially similar to certain dermatomes demarcated by Foerster and that the dermatomes L 1 L 2 and L 3 have retained sweating activity on heating but that the dermatomes lower than this have been deprived of their sweating connections. The paravertebral sympathetic ganglia at these levels, L 4, L 5 S 1 S 2, etc., remained intact in the body but the preganglionic pathway from the spinal cord had been divided when the paravertebral ganglia in the upper lumbar region were removed. After testing by heating this patient was given an injection of a parasympathomimetic drug—carbachol—which induced sweating in those areas which still retained their intact post-ganglionic fibres (Boyd & Monro 1949). It is evident that in this case the ganglion cells in the lower lumbar and sacral segments were uninjured since sweating was found all over the legs but possibly a few ganglion cells have been left behind at operation and supply the skin in the mid-line both front and back, and also in the region of the tenth intercostal nerve on the right side. The rest of the thorax—that is between the fourth thoracic and twelfth thoracic dermatomes—has been deprived of its sympathetic ganglion cells and the post-ganglionic fibres must have degenerated. The sweating in the upper lumbar dermatomes is due to the retention of the intermediate ganglia associated with these particular nerve roots. The position of these ganglia, lying near the inter vertebral foramina, is such that they are not removed by the sympathectomy nor are their pre-ganglionic or post-ganglionic fibres interrupted.

This particular case, in which views are shown of the sides of the body illustrates well the demarcation between the second (and third) lumbar dermatomes and the second sacral dermatome along the dorsal axial line on the outside of the thigh. This line is very constant in position from patient to patient and at successive examinations. The sweat glands just included in the sacral dermatome never appear to be reinnervated from lumbar nerve fibres.

Fig 2 shows patterns observed at intervals up to three years, on case L.C. The lower patterns on the right were those obtained after carbachol sweating The patterns after heating were all very similar but perhaps the first one, observed at only one month, was more extensive as it has not yet reached finality There was also a slight encroachment of sweating in the

After heating

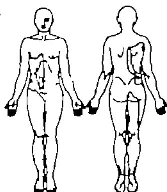
E.1

L. T4–L3 3 weeks
R. T4–L3 8 weeks

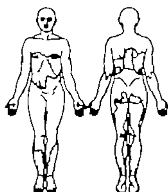
After carbachol

Fig

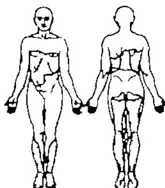
L.C.



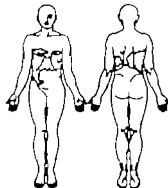
L. Normal
R. T5–L3 1 month



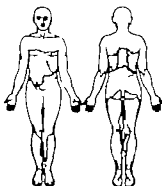
L. T4–L3 3 months
R. T5–L3 4½ months



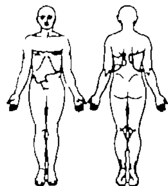
L T4–L3 12 months
R T5–L3 13½ months



L T4–L3 12 months
R T5–L3 13½ months
After carbachol



L T4–L3 35 months
R T5–L3 36½ months



L T4–L3 35 months
R T5–L3 36½ months
After carbachol

Fig.

upper lumbar dermatomes down over the buttock. Carbachol sweating suggested that there was some recovery down the mid-line of the back, but in general, the area of anhidrosis on the thorax at three years was slightly more extensive than that at one and a half years, suggesting that some ganglion cells or their fibres had been involved in scar tissue.

In some patients such as D Cr., whose sweating pattern is shown in Fig 3 sympathectomy was performed for Raynaud's disease of both hands and feet. The usual operations were stellate and second thoracic ganglionectomy for the hands and a lumbar ganglionectomy—usually including the second and third lumbar ganglia—for the feet. After stellate and T 2 ganglionectomy it is to be observed that sweating is retained in the centre part of the face and also in an area in front of the larynx. If carbachol sweating tests are made after such operations as these, anhidrosis may only be found in the regions supplied by the supraclavicular nerves, which have been sectioned in the skin incision, and also in a patchy area often including part of the first thoracic dermatome. With the passage of time, a degree of sudomotor activity often returns to almost all the area originally anhidrotic though occasionally it may be less over the ulnar sides of the forearms.

Lumbar sympathectomy produces a pattern on the legs similar to that found after thoraco-lumbar sympathectomy and this pattern remains remarkably permanent—indeed it is much the same after twelve years.

The perineal region supplied by the lower sacral nerves ordinarily retains its sweating activity and can be demonstrated even shortly after operation and this area may increase very slightly with time.

From these and other cases it can be concluded that after sympathectomy at the appropriate levels, sweating is constantly retained in an area on the face, often over the larynx, constantly in an area involving the L 1–L 2 and usually the L 3 dermatomes, and in the perineum. The areas which remain permanently anhidrotic are the area on the thorax from which the ganglion cells have been removed, and the areas of the lower lumbar and sacral dermatomes, L 4–S 2 inclusive. One striking fact is that these two areas of permanent anhidrosis change so little with the progress of time, indicating that regeneration of post-ganglionic fibres (as on the thorax) or reinnervation from intact fibres (lumbo-sacral) occurs only to a very slight extent. This recovery of function occurs only over the extent of one dermatome at most—such as in the upper thorax region and the abdomen—or on the upper buttock and inner calf where there is slight encroachment from the L 2 into the L 3 dermatomes. There is hardly any spread of innervation where the segmentally successive dermatomes are not contiguous for instance on the lateral side of the thigh between the L 2 and S 2 dermatomes the demarcation remains very precise

These observations lend support to the suggestions of Sperry in regard to the neurones subserving sensation. Sperry (1955) suggested that specific factors in the skin of the embryo are arranged on a dorsi ventral gradient

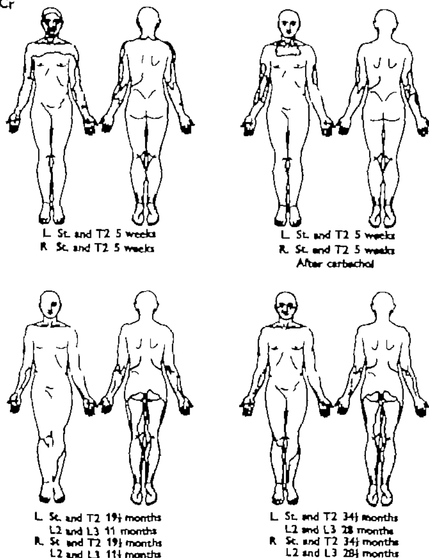


FIG. 3

and thus vary quantitatively whereas on a cranio-caudal gradient they vary qualitatively that is, biochemically Such a conception would seem to apply to the cutaneous autonomic nerves, at least in regard to the post ganglionic neurones. That post-ganglionic fibres are able to reinnervate

the same or adjacent segmental areas of the skin may be seen from a case of brachial plexus avulsion. In Fig. 4, E.C. the area of anhidrosis is shaded whereas the area of analgesia is cross-hatched. Also there is considerable dissociation between the two types of peripheral nervous activity. In this case, however, post-ganglionic fibres are reinnervating or regenerating into the same or adjacent segmental dermatomes and are therefore able to grow long distances to supply the sweat glands in the appropriate area. This case also shows that the area in which sudomotor fibres have established contact with sweat glands, is not necessarily the same as that in which the pilomotor response may be elicited on electrical stimulation (indicated by + sign). For instance, around the shoulder the sweating area is rather more extensive and pilo-erection does not occur where sensation has been regained (absence of pilo-erection is indicated by − sign).

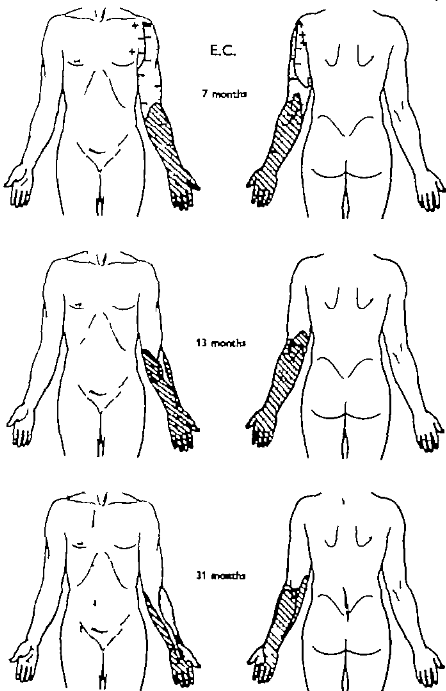
This dissociation of the areas of sudomotor and pilomotor activity had first been observed by Brown & Adson in 1929 who found that, whereas after lumbar sympathectomy pilomotor activity was absent in approximately the same areas as those in which sweating was absent, the pilomotor reflex was present to a variable degree on the shoulders and back where sweating, after cervico-dorsal ganglionectomy, was no longer produced.

Lewis (1930) and Lewis & Landis (1930) showed that after cervico-dorsal sympathectomy the pattern of loss of locally excited pilo-erection did not exactly correspond with that of loss of sweating. Similar observations have been made by Wilkins, Newman & Doupe (1938) and Bickford (1938).

This difficulty in understanding the local pilo-erector reflex was demonstrated in a patient who had undergone a paravertebral sympathectomy but who showed a marked pilo-erector response to stroking—often called dermatographia. The site investigated was on the loin and included an area above which anhidrosis was present on the thorax (ganglionectomized) and below which was an area supplied by the intermediate ganglia (in possibly the T 12 and L 1 dermatomes) which had retained sweating activity.

Within 10 sec. of stroking, pilo-erection appeared across these two areas, thus it occurred in both the sweating and the anhidrotic zones. The flare became apparent after 20 sec., the pilo-erector response still persisting, whereas after 50 sec. the flare was more evident and the pilo-erector response had disappeared (see Monro, 1959).

This pilo-erector response was not seen in the area of skin within the curve of the wound incision and this area was relatively analgesic. On re-examining the patient some months later the analgesia in this area had disappeared and the pilo-erector response had returned. Brodal (1948, p. 377) has also observed this response but thought that it was mediated



L. Brachial plexus avulsion

Fig. 4

only by reflexes passing at the levels of the sympathetic outflow. In this patient, of course, there was no sympathetic innervation to the area that was anhidrotic since all the paravertebral ganglia had been removed as had been shown by testing with carbachol. It is for this reason that I am doubtful whether we should regard pilo-erection as being mediated solely by the autonomic nervous system—if by such we mean the efferent pathway through the pre-ganglionic fibres in the anterior nerve roots. It is still possible that the general pilo-erector response after cooling the whole body may be performed by autonomic nerves but this phenomenon is difficult to investigate even in normal subjects.

In regard to the vasoconstrictor fibres to areas of skin other than the digits, it would seem that their distribution is similar to that of sudomotor fibres. For instance, after thoraco-lumbar sympathectomy in case E.I. (Fig. 1) the skin was livid with dilated and congested blood vessels in the areas that were anhidrotic and there was relative pallor in the upper lumbar dermatomes supplied by the intermediate sympathetic ganglia.

The conclusions drawn from these observations on the sweating and pilo-erector activity in the skin may not be immediately apparent. I have observed a particular patient (W. T.) who had ulcers on the leg above both medial and lateral malleoli, due to severe peripheral vascular disease, and who then underwent sympathectomy at the levels L 1, L 2 and L 3. The ulcer on the outer side, above the lateral malleolus, healed quickly but not so that above the medial malleolus. On testing for sweating activity later it was evident that this ulcer on the inner side of the leg lay within the dermatome (L 3) supplied by intermediate ganglia and that sweating activity and presumably therefore vaso-constrictor activity was retained in this region.

ACKNOWLEDGEMENTS

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THE SENSORY INNERVATION OF THE SKIN

By GORDON H. WRIGHT

One would like to give here a concise and lucid account of the anatomy and physiology of itching and cutaneous pain but, unfortunately at the present time it is not possible to give a concise account, or a lucid one, of any aspect of cutaneous sensibility During the last 100 years and more, much work has been done—clinical histological and experimental but many of the observations have been unsound and for over half a century there has been the baleful influence of two attractive, but mistaken, theories of cutaneous sensibility

For some sixty years the field has been dominated by the theory formulated between 1894 and 1896 by Von Frey that there are four modalities of cutaneous sensation, touch, warmth cold and pain, each type of sensation being mediated by a set of specific sensory receptors nerves, central tracts and brain centres Almost every text book now tells us that hair-endings and *Meissner's corpuscles* are specific for touch, *Ruffini's endings* for warmth, Krause's end-bulbs for cold, and free endings for pain (see Boring, 1942) This theory followed naturally from Müller's so-called law of specific nerve-energies which postulated a single specific function for the fibres of each of the major sense-organs. Similarly Head's idea of twin afferent nervous systems, the protopathic and the epicritic, although repeatedly challenged by many workers since soon after its first publication, still has currency in many text books.

Recent work has shown conclusively that Von Frey's theory is no longer of any use to us in its original form and there has even been a tendency to deny not only the validity of his particular scheme, but also the applicability of Muller's law of specific nerve-energies in any form at all. For a theory of *morphological specificity* one modality one receptor there has been substituted a theory of *physiological non specificity*— any receptor may respond to any stimulus and may contribute to any sensation The death-blow to Von Frey's theory has been delivered largely from a quarter one might hardly expect to be the home of a revolution—Oxford—chiefly I think, from the activities of Dr Graham Weddell and Dr David Sinclair but it is possible that at times they have been led by their enthusiasm into a slight over-statement of their case and, in this matter as in so many others, some of the followers have been more Royalist than the King

It has, therefore, seemed to me that my most useful function would be to present to you, to the best of my knowledge, such *anatomical* facts as seem to command general assent and to be basic to any understanding of cutaneous sensations and perceptions. Such a probing to the depths not only acts as a corrective to the acceptance of any extreme theory it also underlines some basic ignorances. All that can be given is a very brief sketch of a few selected points.

In some recent writings on this subject there has been the suggestion that our physiological theories and interpretations have been straight jacketed by the over-simple vocabulary of skin sensations (Sinclair 1955 Schiller 1956). But, whilst it is true that there are more varieties of sensory experience than there are terms to describe them, it must also be true that only such experiences as are frequently repeated and similar to one another are likely to be given a common name. Whatever theory we adopt, we still have to account for the large numbers of similar sensations we give the name of touch and similarly for pain warmth cold itching and so on.

Three recent reviews have given in some detail the argument against specificity of receptors and pathways, and have proposed as an alternative a pattern theory the possibility is considered that each receptor can respond to any of several different types of stimulus, and that the resulting sensation is determined by the spatial and temporal patterns of the showers of impulses reaching the cerebrum (Weddell, 1955 Sinclair 1955 Schiller 1956). The major anatomical foundations of their case have been provided largely by Weddell and other workers at Oxford the findings have been summarized by Weddell, Pallie & Palmer (1954) An extensive and very careful study of the cornea and of skin from many parts of the body by improved or new histological methods, has shown that many of the appearances recorded by older histologists were artifacts, and that observations made on skin from a few regions of the body have too easily been supposed to apply to all parts of the body

Weddell and his associates have shown that in respect of nerve terminations there are two groups of regions of the body the *hairy* and the *hairless* In the *hairy* regions of the body they have found only two modes of termination —free terminals arranged round hairs, and free terminals in other positions they have found no encapsulated endings in hairy skin. In *hairless* skin and in exposed mucous membranes they have found free terminals and also encapsulated ones in considerable variety Yet they have failed to find any differences in sensibility between the hairy and the hairless regions for instance, in the lip there is no change in sensibility as one crosses the

boundary between hairy skin and smooth mucous membrane. At one stroke, therefore, they have demolished the correlations of Von Frey our task is to review the foundations of a new theory

THE SENSORY INNERVATION OF THE SKIN

The first fact that must be emphasized is the extraordinary complexity of the anatomical pattern of the nerves in the skin. This has been described in detail by Weddell (1941) and by Weddell *et al.* (1954), and also by Cauna (1956). There are two main plexuses of nerves in the skin, a deep corial plexus and a superficial one, the latter being close to the epidermis. In both plexuses there is much branching of nerve-fibres—so much branching that it is impossible to trace a single axon to all of its terminals. Both Weddell and Cauna express the opinion that the terminals from a single axon may occupy an area of 1 sq cm. or more this same area is also innervated by the endings of numerous other axons, forming a very tangled pattern It is unlikely that there is ever any fusion between one branch and another least of all between branches from different axons.

Thus in most instances a nerve-terminal, or receptor is not served by a private line but by a party line (Cauna, 1956). Several or many receptors discharge into a single axon and thus just as there are *motor units* in muscles, so there are *sensory units* in skin.

In their study of the morphology of nerve-endings, Weddell *et al.* (1954) have been able to find only one type

> We have demonstrated that all the cutaneous nerves in the mammalian skin which we have examined end in terminal arborizations of fine naked axoplasmic filaments. Each set of filaments is derived from an ensheathed stem fibre and each filament ends freely in an extracellular position. Thus the actual terminal filaments within the encapsulated nerve-endings appear in no way different from those in the epidermis or those ending in the tissues of a hair follicle

They agree, however that whilst in its *shape* one nerve terminal is much like another it may yet differ markedly in respect of its *position* and in respect of the *calibre* of the nerve-fibre that bears it. The most numerous varieties are as follows.

Pacinian corpuscles are found in many places, such as the mesentery and the fat around the pancreas but in the skin they are found only in the hairless parts and principally at the sides of the fingers, lying rather deeply in the corium or in relation to deeper structures (Cauna & Mannan, 1958). According to Cauna (1956) the axons supplying Pacinian corpuscles leave the branches of the digital nerves before the latter enter the deep corial plexus each Pacinian corpuscle is supplied by a single thick medullated fibre (Cauna & Mannan, 1958). These corpuscles are therefore specific in

position and specific in the manner of their innervation they have often been regarded as receptors for sensations of touch or pressure, but Cauna & Mannan have offered structural evidence that they record the state of openness or closedness of neighbouring blood vessels—an opinion held by many workers during the last 80 years.

Meissner's corpuscles are found in the dermal papillae close to the epidermis, probably only in smooth skin or in exposed mucous membrane, but possibly also in skin with scanty hair Each corpuscle receives from two to nine thick medullated fibres derived from the deep corial plexus they are probably branches from party lines (Cauna, 1956). Weddell *et al.* (1954) agree that the stem fibres to encapsulated endings are of large diameter they also report that some may in addition send branches into the dermis or the epidermis.

Nerve-endings related to hairs have been described in detail by Weddell (1941), for the rabbit. They are in two groups, an outer one in which the endings are around the length of the hair and an inner one in which the endings are parallel to the length of the hair One axon may take part in the innervation of as many as 3000 hairs, and each hair is innervated by terminals from several different axons. These axons are medullated, and some of them are among the largest seen in the skin (Weddell *et al.* 1954).

Epidermal endings are found in all regions of the skin. They are naked terminals, seen between the cells of the deepest part of the epidermis, and it must be emphasized that they are derived from stem-fibres of all sizes. There are also *subepidermal* free endings that are derived from medullated stem-fibres.

Perivascular terminals are found particularly around the arterioles but also in small numbers, around venules they are derived mostly from non-medullated stem-fibres.

Basket like arborisations around sweat-glands are derived both from medullated and from non medullated stem fibres.

Free terminals are found throughout the dermis as well.

There are thus few varieties of *shape* of nerve-ending, perhaps only one, but there is a greater number of *positions* in which the endings can occur and their *positions must determine to some extent the stimuli to which they* are exposed. There are also differences of size and medullation of the various stem fibres that supply branches to the different positions these must determine differences in the conduction rates of the electrical discharges from the endings. We cannot be certain that the differences in the stem-fibres correspond to the differences in the axons (that is, in the nerve trunks), but this may well be the case for in general, at each branching there is some reduction of diameter so that large stem fibres are likely to

be derived from large axons, and small stem-fibres from small axons. Weddell (1941) has stated that the branches of an individual fibre traced throughout its ramifications in the cutaneous plexus bear endings of only one variety.

Sunderland & Lavarack (1953) have marshalled, from the literature many observations which suggest that the axon of a peripheral nerve may branch in almost any part of its course between its distal end and the spinal cord. Such branching must be of the greatest importance in the neurology of cutaneous sensibility and may have a particular significance in respect of pain and itching. Branching would certainly upset the ample textbook scheme of three groups of axons large, medium-sized and small each having specific conduction rates and specific functions. None-the-less Sinclair (1955), one of the chief critics of the older theories, after discussing the many reports of experiments on nerve-blocks made with pressure, cold, and drugs, writes *We must therefore conclude that fibre size is a major factor in the pattern theory of dissociation. For example, it is probable that for a pattern to be recognized as touch it must contain a large proportion of rapidly conducted impulses pain, on the other hand, might require the assistance of many small fibres.*

THE SENSORY PATHWAYS

(a) *Sympathetic system*

Sympathectomy causes no qualitative loss of sensibility in respect of touch or pain or in respect of sensations of warmth (Cooper & Kerslake, 1953) or itching (Rothman, 1941). The evidence as to a *quantitative* effect is conflicting and recently Loewenstein (1956) has demonstrated in the frog a modulation of cutaneous mechano-receptors by sympathetic stimulation. There is a great need for careful comparisons both histological and physiological, between sympathectomized areas and the symmetrically placed areas on the opposite side of the body.

(b) *Dorsal and ventral roots*

It is commonly assumed that the *whole of the cutaneous nerve tangle that remains after sympathectomy is made up of afferent nerves* and it is taken as axiomatic that *impulses from all of these afferent nerves enter the spinal cord by the dorsal roots, rather than by the ventral.* So far as I am aware no conclusive evidence has been offered for either of these beliefs. *Therefore on both counts it would seem to be desirable to test the effects, upon the histological pattern of cutaneous innervation and upon cutaneous sensibility of unilateral section of an extensive series of ventral roots, some time after bilateral sympathectomy.*

(c) *Spinal cord*

Much clinical evidence has accumulated over the last century and more, from cases in which dissociated sensory losses have occurred, that there are distinct pathways for the different modalities of touch, pain, and thermal sensations. But the most clear-cut evidence has come from cases in which the spinal cord has been operated upon for the relief of intractable pain. White, Sweet, Hawkins & Nilges (1950) reported the results from their own series of over 200 cases, and also reviewed the literature there emerged a clear indication that in nine cases out of ten an extensive antero-lateral cordotomy abolishes sensations of pain and temperature without markedly affecting sensations of touch. According to Foerster (1936), Bickford (1938) and Rothman (1941), antero-lateral cordotomy also abolishes all forms of itching

(d) *Brain stem*

Incisions at various levels of the brain-stem, involving the probable positions of the spino-thalamic tract, give similar results to those of antero-lateral cordotomy (White & Sweet, 1955), in respect of pain but there is a greater uncertainty as to the outcome of a tractotomy suggesting a greater degree of individual variation of the pathways.

A total division of the descending (spinal) tract of the trigeminal nerve in the medulla abolishes sensations of pain and temperature in the area of skin that is supplied by the trigeminal, whilst not obviously affecting sensations of touch (Falconer 1949).

(e) *Thalamus*

According to Walker (1938), the majority of the fibres of the spino-thalamic tracts end more posteriorly than the majority of the fibres of the medial lemniscus, in the same thalamic nucleus. This was not entirely borne out by the results of Gaze & Gordon (1954) who recording electrically from single units (but admittedly only a small number of them) in the thalamus of the cat, were unable to find any anatomical separation between the points of arrival of impulses due to small stimuli at the periphery and impulses due to larger stimuli. They did however find, as many others have found, evidence that the pain impulses travel on narrower fibres than the touch ones.

CONCLUSIONS

There is thus a very large volume of evidence that the pathways for touch are distinct and separate from the pathways for sensations of pain and temperature and itching, and there is much evidence that larger fibres,

that are more thickly medullated, are concerned with the conduction of touch impulses, than is the case for pain. It is hard to escape from some degree of specificity

The time has now come to quote, I hope without misrepresentation, three short, cautious statements from the writings of the Oxford School.

We can now suggest that nerve-terminals as such can only be specifically related to sensory modalities in respect of their situation in the skin relative to the stem fibres from which they arise, bearing in mind the influence of their chemical environment (Weddell *et al.* 1954).

It is of interest that some of these [free] terminals are situated among the arterioles, arteriovenous anastomoses, and capillaries of the skin, positions highly suitable for thermometric purposes (Weddell *et al.* 1954).

It appears that the terminal subserving pain is a fine freely-ending arborisation of nerve fibres. But it is possible that a different pattern of impulses from these same terminals, either alone or combined with impulses from hair terminals or nerve end-corpuscles, may produce other sensations (Weddell & Sinclair 1953).

Thus both those who still think that Müller's law of specific nerve-energies may apply to the cutaneous afferent system, and those who do not, agree that large groups of nerve-terminals differ in respect of their positions in the skin in respect of the diameters and degrees of medullation of the axons that they discharge into and in respect of the central pathways into which they debouche. It is also hard to escape from the evidence that these anatomical differences correspond to some degree of physiological division of labour

May it not be possible that, in our dissatisfaction with the now-evident crudities of the Von Frey theory and our enthusiasm to debunk it, we have thrown out the baby with the bath water? Perhaps we have expected too much of Von Frey's ideas, and indeed have expected too much of the peripheral nervous system. Maybe in the past we have tried to explain, by reference to features of the histology of the cutaneous receptors and plexuses, functions which are performed centrally probably in the cerebral cortex. As an example may I cite some recent work done in this Department, on tactile localization? In this work it was shown that there is an interplay of tactile, thermal proprioceptive and even visual factors in tactile localization—an interplay that can only occur centrally (Halnan & Wright 1959). We must always be mindful of the difference between sensation and *perception*. The problems of cutaneous sensibility are enormously complicated, all too few people have worked upon them, and much more needs to be done, both physiologically and histologically I am quite sure that a lot of this work will necessitate a much fuller consideration of the cortical factors involved.

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PLASMA KININ FORMATION AS A FACTOR IN ACTIVE VASODILATATION IN SKIN

By R. H FOX and S M HILTON

Active vasodilatation is the term used to describe the opening of the vascular bed, with its concomitant increase in blood flow caused either by specific vasodilator nerve fibres or by any mechanism forming or releasing vasodilator substances in the tissue itself It is to be contrasted with the passive vasodilatation which occurs when vasoconstrictor tone, ordinarily maintained by the activity of specific vasoconstrictor nerves, is lessened or removed. In other words, active vasodilatation results from an increase in nervous activity whereas passive vasodilatation is the result of a decrease in nervous activity

Heating one part of the body for example the legs, produces a vasodilatation in the unheated parts, such as the arms and hands, which is in part active and in part passive. The vasodilatation in the hand is certainly very largely the result of inhibition of vasoconstrictor tone, for if the peripheral nerves to the hand are blocked with a local anaesthetic there is an immediate and large increase in hand blood flow (Lewis & Pickering 1931) Some workers had originally assumed that the skin of the rest of the body would be like that of the hands, having a vasoconstrictor mechanism of control, but a few years later Grant & Holling (1938) published evidence, based on measurements of skin temperature, for an active vasodilator mechanism in the proximal parts of the limbs which is brought into play by body heating Recently their findings have been confirmed using the more direct method for measuring blood flow of venous occlusion plethysmography (Edholm, Fox & Macpherson, 1956 1957) and by other workers who observed the changes in oxygen saturation of venous blood (Roddie Shepherd & Whelan, 1956 1957).

It will perhaps be useful to recapitulate briefly some of the evidence described by Edholm *et al.* (1956 1957) for an active vasodilator mechanism in the forearm skin. Using the venous-occlusion plethysmograph to measure forearm blood flow it was first shown that the increase in blood flow through the forearm during indirect heating of the body is confined entirely to the skin and does not extend to other tissues such as muscle. This was done by the iontophoresis of adrenaline into the skin of one of the subject's forearms, thus virtually abolishing the skin blood flow and then observing the changes in blood flow in the two forearms during body

heating In such experiments it was found that iontophoresis of adrenaline into the skin can abolish the forearm vasodilatation, indicating that it is confined to the skin (Fig 1)

Information showing that this vascular change is an active vasodilator process resulting from an increase in nervous activity was obtained by measuring the forearm blood flow before and after blocking the cutaneous nerves with a local anaesthetic. With the subjects in a neutral thermal state, that is to say feeling neither warm nor cool, the cutaneous nerves to one

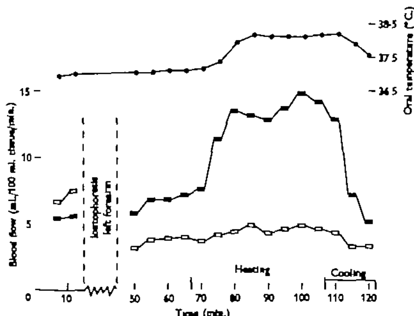


Fig. Blood flow in both forearms (right ■ left □) before and after adrenaline iontophoresis of the left forearm and the response to body heating and subsequent cooling The oral temperature is also shown.

forearm were blocked and the rates of blood flow through the normal and blocked forearms were then found to be on the average the same (Fig 2). These experiments showed that the low blood flow in the forearm skin of a subject in the neutral thermal state is not the result of a high vasoconstrictor tone, for if it were the blood flow would have been much higher on the nerve-blocked side, as indeed is found, for instance, when a similar experiment is performed on the hand. Furthermore, although the mean blood flow is the same in both blocked and normal forearms, the range is less on the blocked side. This is evidence for the presence of both small amounts of vasodilator activity in some experiments and of vasoconstrictor activity in others. At present we know very little about the functions of

the vasoconstrictor nerves in the forearm skin, except for this negative information that they play only a very small part in the vasodilatation of body heating They may play some part in closing down the circulation in a subject whose skin is in the vasodilated state and who is then suddenly exposed to a strong cold stimulus, for it has been shown that the vasodilatation resulting from the application of a rubefacient ointment containing a nicotinic acid ester can be abolished by exposing the subject's body to a cold stimulus (Crismon, Fox, Goldsmith & Macpherson, 1959)

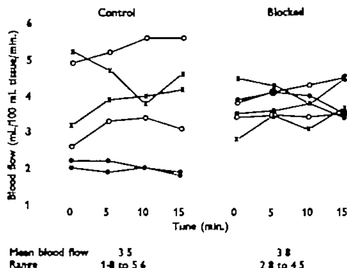


Fig 2. Comparison of the blood flow in control and cutaneous nerve-blocked forearms of three subjects on two occasions in constant and comfortable environment

The effect of blocking the cutaneous nerves to forearm skin on the forearm blood flow during body heating is shown in Fig 3 The blood flow in the untreated forearm shows a normal increase with heating whereas on the blocked side the increase is virtually abolished. Furthermore the response is absent in patients who have had a sympathectomy (Barcroft & Swan, 1953). It can therefore be concluded that the forearm vasodilatation accompanying body heating is the result of an active vasodilator *mechanism* in the skin, mediated by sympathetic nerves.

For a long time there has been discussion and argument as to whether there are indeed any active sympathetic vasodilator nerves. Evidence has been presented, mainly in animal experiments, for or against such nerves to skeletal muscles, to the intestines to the coronary vessels, to the salivary glands and to certain skin areas. However the vasodilator nerves to skeletal muscle are the only firmly established contenders for this title at the present time (Uvnäs, 1954) The fate of the supposed vasodilator nerve supply to

the salivary gland is of particular interest to us here because the connection between salivary gland activity and vasodilatation in the gland is probably the same as that between sweat gland activity and the active vasodilatation which results from indirect body heating, in the skin. It used to be thought that there must be vasodilator nerve fibres in the nerve to the salivary gland and that these were responsible for the functional hyperaemia in the gland which accompanies its activity. The main reason for this belief was

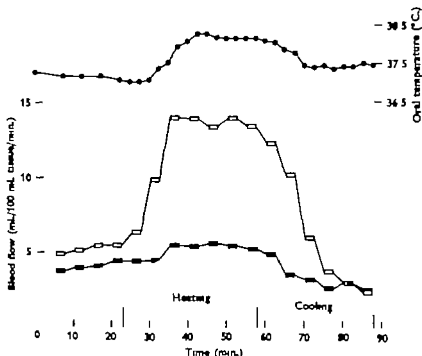


Fig. 3. Blood flow in both forearms (right ■, left □) after cutaneous nerve block of the right forearm and the response to body heating and subsequent cooling. The oral temperature is also shown.

the discovery that stimulation of the chorda tympani to the atropinized salivary gland fails to produce extrusion of saliva but nevertheless causes an increase in blood flow. So it was said that there must be two different sets of nerves, one which causes secretion of saliva and another which is responsible for the vasodilatation. Recently this problem was re-examined by Hilton & Lewis (1955*a*, *b*, 1956) who showed that when stimulation of the secretomotor nerve activates the salivary gland a specific enzyme is released not only in the saliva but also into the surrounding tissue fluid. There, it acts on the pseudoglobulin fraction of the tissue fluid plasma proteins to form a powerful vasodilator polypeptide called plasma kinin.

The plasma kinin is in direct contact with the vessels supplying the gland and vasodilatation results. It is clear therefore, that the secretory function is in fact more sensitive to atropine blocking than is the function of releasing the plasma kinin enzyme. It should be said at this stage that there are several such plasma kinins described in the literature, though they have been given different names. They are all polypeptides which are either identical or at least very closely related compounds. All are vasodilator and smooth muscle stimulating substances which are formed by the action of enzymes on the pseudoglobulin fraction of plasma proteins. One such smooth muscle stimulating polypeptide was obtained by incubation of snake venom or trypsin with serum, plasma or fractionated plasma globulin, and this was called bradykinin (Rocha e Silva, Beraldo & Rosenfeld, 1949). Then a similar or perhaps identical, polypeptide was obtained by the action of tissue extracts on plasma proteins and this was called kallidin (Frey Kraut & Werle 1950). The different names, of course do not necessarily indicate any difference in structure, and it was to overcome this confusion that the general title of plasma kinin has recently been suggested.

This work on the relation between secretion and vasodilatation in the salivary gland suggested a possible explanation for the vasodilator mechanism in forearm skin (Fox & Hilton, 1958). There are a number of similarities between the eccrine sweat glands and the salivary glands, and it therefore seemed possible that the sweat glands might also produce a plasma kinin forming enzyme and that this, rather than specific vasodilator nerves, might be the cause of the active vasodilatation in forearm skin. Samples of human sweat were analysed and it was found that the plasma kinin forming enzyme is indeed present in sweat as it is in saliva. During a prolonged period of continuous sweating it was found that the concentration of the enzyme in the sweat first increased and then decreased, but was still easily detectable at the end of 90 min. (Fig 4). The important point is the presence of the enzyme in human sweat, for this proves that this vasodilator polypeptide forming mechanism is present in the sweat glands the observed changes in the concentration of the enzyme in the sweat do not necessarily reflect the changes that are occurring in the activity of the polypeptide in the tissues. In fact, the vasodilatation in the forearm skin usually precedes the onset of detectable sweat secretion. In an attempt to obtain further information on the activity of this mechanism in the skin itself experiments were performed in which a localized area of the subcutaneous space of the skin of the forearm was perfused with saline and the plasma kinin activity of the resultant perfusate was measured before and during a period of active vasodilatation (Fig 5). It was found that there was an increase in the amount of active polypeptide present in the perfusate during the period

of active vasodilatation and that this increase preceded the onset of sweat secretion (Fig 6). A simple schematic diagram showing the suggested mode of action of this plasma kinin system in human skin is shown in Fig 7 There is further support in favour of the analogy between the vasodilatation

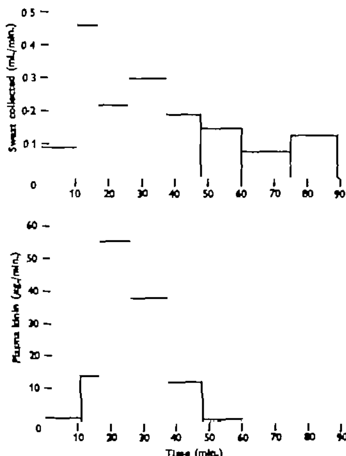


Fig. 4 Histograms of the amounts of sweat collected from the human forearm during period of body heating and of the corresponding plasma kinin forming activity of each sample.

in the salivary gland and that in the forearm skin. The forearm vasodilatation is atropine-resistant like that of the salivary gland. A dose of atropine which is sufficient to suppress sweat secretion delays but does not prevent the vasodilatation.

As mentioned already the active vasodilatation in human forearm skin can precede the onset of sweating, particularly when the subject is only exposed to a low heat stress. In experiments on the cat's submandibular

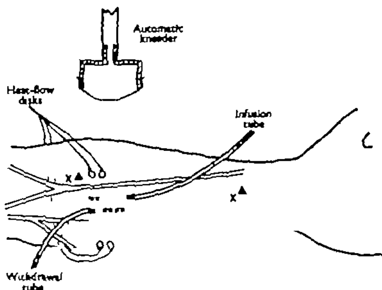


Fig. 5. Diagram showing sites of perfusion of subcutaneous space and measurement of heat flow skin temperature (×) and skin resistance (▲). Automatic kneader shown in cross-section.

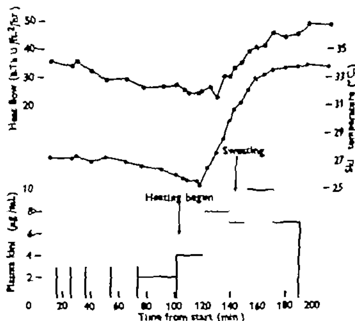


Fig. 6. Results of an experiment in which the subject was heated after control period of cooling, showing measurements of forearm skin temperature (●), heat flow (O) and equivalent plasma kinin content of successive samples of perfusate from the subcutaneous space.

salivary gland it was similarly shown that whereas with maximal stimulation of the chorda tympani vasodilatation and extrusion of saliva are observed simultaneously with submaximal stimulation vasodilatation can appear before, or even without, extrusion of saliva. Another important point is that animals like the cat and dog, which lack eccrine sweat glands, do not have an active vasodilator mechanism in the skin, and the vasodilatation in the skin occurring with heating is the result of the release of vaso-constrictor tone (Folkow Frost, Haegar & Uvnäs, 1949 Green, Howard & Keenan, 1956).

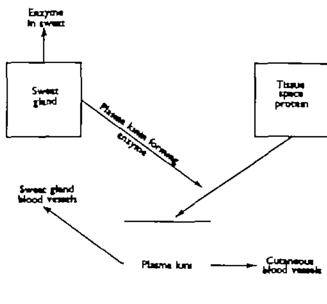


Fig. 7 A schematic diagram to illustrate the plasma kinin mechanism in human skin.

From evidence such as has been cited indicating that active vasodilatation in human forearm skin is secondary to sweat gland activity we cannot exclude the possibility that there are, in addition, sympathetic vasodilator nerves to the blood vessels in the skin of the forearm. It is quite possible that the mechanism involved is a great deal more complex than at present envisaged. Before we can settle such questions finally we need to know more about the pharmacology of the plasma kinins and we must find ways of blocking or potentiating their actions *in vivo* Nevertheless, the concept that sweat gland activity may control both the major mechanisms regulating the thermal exchanges of the body—cutaneous vasodilatation and sweating—seems both efficient and reasonable. The fact that the plasma kinin forming mechanism exists in human skin, and that it is an important

factor in the physiological mechanism of heat vasodilatation, suggests that it could play a role in pathological processes also The H-substance of Sir Thomas Lewis, which is formed by mild trauma to the skin, was never thought to be a single substance (Lewis, 1927) and one of its components is known to be relatively non-diffusible Krogh (1929) suggested the name H-colloid for this factor This could be plasma kinin or the enzyme forming it. In support of this, mild burning of the skin is known to release proteolytic enzymes of the type which could form plasma kinin from plasma proteins (Beloff & Peters, 1945) Malfunctions of this system may also play some part in the production of dermatological disorders. Herxheimer (1956) for instance, has observed that the skin reaction of cholinogenic urticaria is always associated with both sweating and vasodilatation, and he therefore suggested that this reaction was secondary to some product of sweat gland activity However the many problems raised by these observations, which are probably of more immediate concern to the practising dermatologist, remain to be resolved.

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ACETYLCHOLINESTERASE RESPONSES IN PHYSIOLOGICAL AND PATHOLOGICAL VASOMOTOR REACTIONS

By A. SCOTT

INTRODUCTION

The structural background of the blood supply to the skin was well outlined by Spalteholtz. Large arterioles entering from the subcutis anastomose in the deeper dermis, and smaller arteriolar branches pass superficially to a further anastomotic plexus in the superficial dermis. Metarterioles from this layer are distributed to the papillae in which they merge into capillaries through precapillary sphincters. All the arterial channels mentioned with the exception of the capillaries have muscular components in their walls. The venous return flow passes similarly through several anastomotic layers of venules and small veins into the larger veins of the deeper dermis.

The cutaneous nerves are broadly separable into those belonging to the peripheral nervous system—entirely sensory to the skin, and those from the autonomic nervous system which appear as far as is known at present, *to be efferent only in function. Von Frey first described the sensory* terminations as specialized end-organs in the superficial dermis. However the more recent studies of Woollard & Weddell have demonstrated that in all but the special skin surfaces such as the hand, lip, etc., most of the nerve terminations are in the form of free endings or networks of fibrils in the superficial dermis and epidermis. As yet no parasympathetic nerves have been observed in the skin, so that the efferent autonomic fibres are representative of the sympathetic outflow only These fibres supply all vessels having a muscular coat, plus the epidermal appendages including the pilosebaceous apparatus (adrenergic fibres) and the eccrine and apocrine sweat glands (cholinergic and adrenergic fibres) No afferent sympathetic nerves have yet been demonstrated in the skin.

PHYSIOLOGICAL INTERRELATIONSHIP OF BLOOD SUPPLY AND NERVES

Vasoconstrictor fibres are carried by adrenergic sympathetic nerves to all arterioles, metarterioles and precapillary sphincters, that is to those vessels possessing a muscular coat. The fibres do not extend beyond the limit of the last named structures. The constrictor stimuli thus delivered are not constant in intensity but provide a rhythmic tonus in the arterioles.

The existence of vasodilator fibres is still the subject of controversy despite many experiments designed to provide evidence of their presence. However the consensus of opinion is against their existence as such, although the most recent work of Grant suggests that some vasodilator fibres function in the rabbit ear. Failing the acceptance of sympathetic vasodilatory fibres, one must seek in another direction for an explanation of peripheral cutaneous vasodilatation. The only other nerve fibres obviously represented in the skin are the afferent sensory nerves of the peripheral system. The concept of antidromic vasodilatation was first introduced by Lewis. This involves fibres of the sensory nerve—either identical with those transmitting sensory impulses or fibres separate in function but anatomically associated with the sensory nerve—in either case having their neuronal stations within the dorsal root ganglion. Closely associated with this functional neuronal entity is the axon reflex arc of Langley again fibres either identical with, or closely associated with, the sensory nerve are utilized as afferent and efferent arms of a reflex arc whose ganglion is the most distal point of ramification of the nerve being activated. Such an axon reflex is similar in concept to the sympathetic axon reflex described in relation to sweat glands.

As yet the transmitting substance of sensory impulses has not been conclusively identified. It does not appear to be acetylcholine or histamine, although the former substance will discharge sensory end-organs. ATP has been suggested as the functional agent by Holton & Holton (1954) although materials such as the dorsal root ganglion extract of Hellauer & Umrath (1948) are still being investigated. Whether the sensory transmitter is also concerned with the passage of vasodilator impulses is again unknown. Various workers have demonstrated acetylcholine (Wybauw 1938) in perfusates of tissue after stimulation of these antidromic fibres, while others have been able to obtain substantial quantities of histamine (Von Euler & Purkhold, 1951). Most of the difficulties arise when comparing the type of peripheral dilatation obtained with these materials with that naturally observed. If such a criterion were valid then both ATP and the dorsal root extract would appear to be more suitable choices for the function of transmission.

These two mechanisms are useful in explaining the active participation of muscular arterioles in vasodilatation, but cannot be directly concerned with the dilatation of capillaries which lack a nerve supply. Can these vessels undergo active alteration in size or is this change merely secondary to the haemodynamic effect of the blood flow controlled by metarterioles and precapillary sphincters? Chambers & Zweifach (1944) describe a spontaneous change in size apparently due to imbibition of water by individual endothelial

cells, thereby constricting the lumen corresponding loss of fluid results in flattening of the cells and dilatation of the lumen. Such observations suggest that the size of the capillaries is closely linked to the degree of their permeability If this is the case then the local concentration of materials which might affect their permeability assumes importance. Capillaries react quickly to the presence of local metabolites such as carbon dioxide, lactic acid, etc., and to local hormones such as histamine, acetylcholine, nor-adrenaline. The latter two can both be derived from nerves and blood vessels in the vicinity as can their respective enzyme systems, while histamine has been identified in the cells of the basal epidermis. Our investigations were undertaken to determine some of these points.

The groups studied included normal controls, patients with psoriasis (representing cases with a dermatosis and normal skin reactions), patients with subacute and chronic eczema, and patients with the so-called atopic syndrome (the latter two groups representing cases with temporarily and permanently altered skin responses respectively) The test materials included acetylcholine and histamine as the two vasodilators, trafuril as a secondary dilator and various possible inhibitors, such as atropine, nicotine, eserine, ansolysen. Our methods of observation involved gross visual examination, the use of the capillary microscope, and the demonstration of acetylcholinesterase in biopsies of tested skin, using a modification of Koelle s method of histochemical stain.

Table 1 *Vasomotor reactions to locally introduced chemicals*

	Percentage of patients with vasodilatation responses					
				Atopic syndrome		
	Normal	Psoriasis	Eczema	I	II	Combined
Acetylcholine	00	00	00	00		50
Eserine	00	00	28	60	0	30
Histamine	00	00	47	33		17
Trafuril	00	00	47	33		7
Atropine	00	00	33	73		37
Nicotine	0					

In the normally reactive skin (including the psoriatic skin) vasodilatation is observed in response to heavy stroking, the intradermal injection of acetylcholine, histamine, eserine, atropine, and the external application of trafuril, a histamine-releaser No reaction results from the injection of saline apart from a transient erythema at the site due to capillary dilatation. Induced active vasodilatation involving small arterioles begins approximately 2 min. after the injection (4 min. after application) and reaches its maximum within 5 min. Duration may be as long as half an hour but is usually about 15 min The capillaries and sterioles participate in the response.

This visible reaction is associated with histochemical evidence of the presence of acetylcholinesterase in the superficial nerve endings of the test area, and in and about the walls of the small arterial channels including the capillaries.

Of the two groups with abnormal skin responses, those with eczema failed to develop vasodilatation with one or more of the test substances, although all showed a persistently normal response to local acetylcholine. Where no dilatation was visualized either grossly or with the capillary microscope, no acetylcholinesterase could be demonstrated in the expected sites. One particular group with the atopic syndrome (comprising 50 per cent of them) despite the absence of any eczematization, failed to develop gross vasodilatation even with acetylcholine. However microscopically acetylcholinesterase was still identifiable in the cutaneous nerves and about the blood vessels.

Table 2. *Presence of cholinesterase in the skin*

Diagnosis	Flare of triple response	Cholinesterase around arterioles	Localization in nerves
Normal	Present	Present	Present
Psoriasis	Present	Present	Present
Eczema			
Active	Absent	Absent	Absent
Healing	Present	Present	Present
Atopic syndrome			
Group I			
Active	Absent	Absent	Absent
Healing	Present	Present	Present
Group II			
Active	Absent	Absent	Absent
Healing	Absent	Present	Present

Attempts to prevent this dilatation in the normal can be grouped into methods which block local cellular responses—atropine thus failed to inhibit either the gross visible response or the microscopic appearance of the enzyme. Secondly those which block local nerve responses—nicotine prevented both gross and microscopic reactions, and sensory nerve degeneration resulted in a similar inhibition, while sympathetic nerve degeneration failed to effect any alteration. Thirdly interruption of local pseudoganglion activity using local anaesthesia prevented the reactions, while ansolysen did not. Root anaesthesia, which blocked the connection of the dorsal root ganglion with the periphery did not affect the responses. Finally substances which alter local permeability such as the steroids and hyaluronidase, did not cause any variation in response.

On the basis of these observations it appears that in the normal response vasodilatation depends upon the integrity of local sensory nerve fibres at least up to their most distal point of ramification and is closely associated with the release of acetylcholinesterase from these nerve endings, from

which it appears to diffuse to adjacent small vessels. This release is usually detectable prior to the visible reaction.

When one considers the form of response in the patients with abnormal reactions and eczema, one must assume the integrity of these same nerve pathways both from the usual clinical examination and from the persistently *normal* response to acetylcholine. We were unable with any of our test methods to restore these responses to normal. (No steroid was used since it would obviously clear the eczema.) This would suggest that the blood vessels are still capable of reaction, but are sufficiently under the influence of local metabolites associated with the eczematous process that they can no longer respond to physiological concentrations of any of the test substances with the exception of acetylcholine.

Table 3 *Alterations in vasomotor responses in the skin*

Agent	Normal	Eczema	Atopic II
		Visible effects	
Adrenaline	N change	No change	No change
Atropine	No change	N change	Becomes normal
Nicotine	Blocks all reaction		
Hexamethonium			
Low concentration	N change	N change	No active constriction
High concentration	Causes dilatation of vessels by itself		
Hyaluronidase	Increases dilatation	No change	Becomes normal
Hydrocortisone (Locally—2 wk.)	N change	No change	Becomes normal
Sensory nerve degeneration	N visible reaction		
Sympathectomy	No change	No change	No active constriction
		Alteration in cholinesterase	
Adrenaline	N change	No change	N change
Atropine	N change	N change	N change (present)
Nicotine	N enzyme present		
Hexamethonium			
Low concentration	No change	No change	N change
High concentration	N change	No change	No change
Hyaluronidase	N change (present)	No change (absent)	N change (present)
Hydrocortisone (Locally—2 wk.)	N change	No change	N change
Sensory nerve degeneration	No enzyme present		
Sympathectomy	No change	No change	N change

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PLATE I



Fig.



Fig.



Fig 3



Fig 4

For explanation see p. 98

(*Facing p.* 96)

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATE

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Fig. Section of skin showing acetylcholinesterase in nerves in the superficial dermis and papillae, with deposits adjacent to capillaries. × 50.

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DISCUSSION

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INGRAM. Dr Weddell impressed upon us the pattern of the innervation of the skin and the effect of damage. We are constantly faced with injuries of the skin and the effects of such injuries must be considerable on the superficial nerves. We know from the experiments of Holti (1955) and others how long recovery is delayed after damage to skin vessels and I presume the same happens with nerves. I also assume that this varies a good deal from individual to individual. The thing that is borne in upon me is the long time that it takes for the skin to get back to normal physiological function and structure and its bearing upon prognosis and our advice to patients with industrial dermatitis and eczema. We are grateful to the openers for their stimulating papers.

Dr C. D Calnan (London). I would like to ask whether there is any evidence from embryology or comparative anatomy as regards the Meissner's corpuscles and the Pacinian corpuscles which are found in man but not regularly distributed all over the skin. Is there any evidence that these have any function now in man at all, or whether they are anachronisms? If the complex pattern of innervation to hairs on the rabbit's ear as shown by Dr Weddell, is present in man, does stimulation of hair play any part in man s sensations? If one loses hair as in cases of alopecia totalis it does not seem to affect sensation at all Is it possible that the skin is dependent for its sensation merely on the terminal nerve endings?

Dr G Weddell (Oxford) The Meissner corpuscles in hairless skin are important, multiply innervated tactile end-organs. In my opinion they are the counterpart of hair follicle and end-organs in hairless skin. In this connection it is of interest that in skin which has become bald there is no detectable change in tactile acuity yet many of the hair follicles have collapsed and their neural elements have come to resemble those in Meissner corpuscles very closely

It is possible to distinguish two quite different types of nerve termination in the skin. Encapsulated end-organs which include hair follicle end organs, Meissner and Pacinian corpuscles. Essentially these consist of neural elements lying in specific relationship to compact groups of specialized cells of extra-neural origin and each end-organ has a range of forms. They are served by nerves of large diameter which enter the dorsal columns of the spinal cord. In sharp contrast are diffuse arborizations of filaments which appear to terminate freely throughout the tissues of the skin they are served

which it appears to diffuse to adjacent small vessels. This release is usually detectable prior to the visible reaction.

When one considers the form of response in the patients with abnormal reactions and eczema, one must assume the integrity of these same nerve pathways both from the usual clinical examination and from the persistently normal response to acetylcholine. We were unable with any of our test methods to restore these responses to normal. (No steroid was used since it would obviously clear the eczema.) This would suggest that the blood vessels are still capable of reaction, but are sufficiently under the influence of local metabolites associated with the eczematous process that they can no longer respond to physiological concentrations of any of the test substances with the exception of acetylcholine.

Table 3 *Alterations in vasomotor responses in the skin*

Agent	Normal	Eczema	Atopic-II
		Visible effects	
Adrenaline	N change	N change	No change
Atropine	N change	N change	Becomes normal
Nicotine	Blocks all reaction		
Hexamethonium			
Low concentration	No change	No change	No active constriction
High concentration	Causes dilatation of vessels by itself		
Hyaluronidase	Increases dilatation	No change	Becomes normal
Hydrocortisone (Locally—2 wk.)	N change	No change	Becomes normal
Sensory nerve degeneration	N visible reaction		
Sympathectomy	No change	No change	No active constriction
		Alteration in cholinesterase	
Adrenaline	N change	No change	N change
Atropine	N change	N change	N change (present)
Nicotine	N enzyme present		
Hexamethonium			
Low concentration	N change	N change	No change
High concentration	No change	No change	No change
Hyaluronidase	No change (present)	No change (absent)	No change (present)
Hydrocortisone (Locally—2 wk.)	N change	N change	No change
Sensory nerve degeneration	N enzyme present		
Sympathectomy	N change	No change	No change

There remains the group of patients with the atopic syndrome who though free of any eczematous process still failed to show a visible vasodilatation in response to any of our test materials. It is significant that they failed to respond to acetylcholine as well. However it was observed that

the prior use of certain materials did result in the development of the normal response. Chief among these was hyaluronidase and the local steroids, both of which alter permeability albeit in opposite directions, and relieve local oedema. When one recalls the presence of acetylcholinesterase in the microscopic sections from these cases, despite the absence of visible dilatation, it is not surprising to find as we did that merely dispelling the oedema sufficed to make visible the dilatation normally expected. This can actually be visualized with the capillary microscope.

The acetylcholine response in the atopic must be dealt with separately since an active vasoconstriction developed in this group, in contrast to a mere absence of dilatation. Although originally inclined to attribute this unusual reaction to oedema as well, I now doubt that this is so, particularly since the pallor has a delayed appearance, and its development is prevented by the prior use of atropine. We were able to find only one atopic patient who had had a sympathectomy and here no vasoconstriction was observed. In consequence it might be suggested that this vasoconstrictive effect of acetylcholine is due to activation of a local sympathetic axon reflex with release of adrenergic impulses to the blood vessels effecting an active vasoconstriction. Such a suggestion requires more investigation.

In summary then, it has been established that the flare of the triple response and the flare induced by intradermal chemicals is produced by the local activation of axon reflexes involving sensory nerve endings only (subject to the proof of separately existent antidromic fibres)

The dilatation so produced is associated with the release of acetyl cholinesterase from superficial nerve endings and its presence in and around the walls of local small blood vessels including capillaries, either by means of its production in the vessel, or more likely through diffusion from nerves.

In the absence of dilatation, as in the eczematous subject, no acetyl cholinesterase is released. Although no dilatation is ordinarily visible in atopic patients, it can be visualized by the use of hyaluronidase, and the capillary microscope, and is always associated with the release of acetyl cholinesterase.

In view of the presence of this enzyme, and its known function in acetyl choline catabolism, it is suggested that acetylcholine itself is released from nerve endings, to function as a local hormone in the production of vasodilatation through its action on the small blood vessels after diffusion.

ACKNOWLEDGEMENTS

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by thinner nerves which terminate in the antero-lateral columns of the spinal cord. In lower vertebrates such as fish, there are no encapsulated end-organs served by spinal nerves in the skin and it is of interest that the dorsal columns of the spinal cord are poorly developed. These two morphologically contrasting systems are also sharply differentiated physiologically. For instance, neither warming nor cooling evokes a discharge of action potentials from hair follicle end-organs yet the slightest movement of a hair gives rise to an outburst of activity.

INGRAM. I would like to ask about the Langerhans cell. Is it an effete melanocyte or a nerve structure?

WEDDELL. The Langerhans cell is most probably an effete melanocyte. The holocrine secretory process to which I referred earlier is thought to take place as follows. Menoblasts give rise to melanocytes, which in turn give rise to Langerhans cells which in due course are shed together with keratin.

PROFESSOR J. D. BOYD (Cambridge). I agree.

DR G. C. WELLS (London). Dr Fox's work is extremely interesting and I would like to hear more about the kinins. Are they to be found in other glands or organs besides the salivary and sweat glands already mentioned?

INGRAM. I presume there are other stressor agencies than heat which apply here.

DR R. H. FOX (London). The enzymes forming plasma kinins are present in the secretions of many of the glands which produce a profuse watery secretion. They are to be found not only in sweat and saliva but also, for example, in pancreatic juice and urine. G. P. Lewis (personal communication) has found that the plasma kinin forming enzymes are also present in skin which has not got eccrine sweat glands. Plasma kinins can also be formed by blood plasma, so that these substances do have a wide distribution and significance. There must be some differences in structure, though what they are we do not know at present. I should like to leave further discussion on this to Schachter who is going to talk to you later in the week.

We do not know whether this mechanism plays a part in types of vasodilatation other than that caused by body heating. We cannot even say that there are no active vasodilator nerves present in forearm skin. The action of plasma kinins could, however, play some part in the vasodilatation of local heating because we know that local heating can activate the sweat glands to secrete sweat.

DR F. A. WHITLOCK (Newcastle). Would Dr Wright amplify his statement that sensation could be modified by stimulation of the sympathetic nerve supplying the area from which the sensation is arising? This observation suggests affinities with the work of Grant (1955) and I wondered what sensation was being experienced and how it was altered.

DR G H. WRIGHT (Cambridge). Loewenstein (1956) showed that the pattern of impulses in an afferent nerve from the skin could be altered by stimulation of the sympathetic nerves to the same part of the skin. But his work was done in the frog, so we can know nothing about the associated sensations. Indeed, I would like to emphasize the probability that cutaneous afferent nerves can sometimes be fired off without any sensation at all being evoked.

DR P A. G MONRO (Cambridge). In Dr Fox's first diagram of the production of sweat and corresponding concentrations of plasma kinin, sweat production, on heating, went up to double or treble the resting level, and then remained fairly steady Production of plasma kinin was delayed, but later rose to a peak and then seemed to fall off almost to zero again. I would be interested to know whether the sweat secreted later in the experiment contained only little plasma kinin, and if so, would Dr Fox comment on this.

FOX. The changes in the amount of enzyme in sweat and in saliva are probably not very helpful because it is enzyme which is escaping in the wrong direction and we may be merely looking at a washing-out process. We should not necessarily assume that the decline in concentration is evidence of fatigue. It is possible that fatigue or dysfunction of this mechanism may be playing some part in the skin conditions found in heat illnesses, for example prickly heat. May I take this opportunity to add that if anyone has a case of congenital ectodermal dysplasia with absence of sweat glands we shall be very *interested to investigate the vasomotor* responses of such a subject.

DR J MARTIN BEARE (Belfast). Many of us have an idea that senile pruritus is due to changes which take place in the central nervous system and not to small changes in the skin.

WEDDELL. I have no views. I have examined specimens of skin from patients with senile pruritus but with the techniques at my disposal I have not been able to detect any neuro-histological abnormalities in this disease. The implications of what Dr Wright has said are most important. For instance, because of the phenomenon of facilitation, morphologically similar networks may have come to behave quite different physiologically Also it is important to remember that paraesthesiae may result from a disturbance anywhere between the skin and the many parts of the brain responsible for perception. I often formulate ideas about the role of the peripheral nervous system in sensory perception, but as time passes I am less inclined to make any dogmatic statements because I am beginning to know how complicated the peripheral mechanisms are and I am aware that far more circuits must be activated centrally than peripherally All I do

know is that in response to stimulation of the skin the peripheral nerves throw a highly complex pattern on to the spinal cord. What happens then is fortunately outside my field!

DR H R. VICKERS (Oxford) Can irritation ever be initiated centrally? Why I ask is that I have been looking out for patients who have had an amputation, with a phantom limb who have had generalized irritation and they never have complained to me of irritation in the phantom limb. I have asked them specifically whether the phantom limb has ever itched and it never has. One felt that *itching was a purely peripheral concept* that could not be induced centrally as suggested by Dr Martin Beare.

WELLS. I have been seeing a young man who had had a leg amputated after a motor-cycle accident. He had no history of past skin disease, but he developed infected eczema above the stump and this was followed by intensive nummular spread and much itching He volunteered that one of the things which bothered him most when he was sitting by the fire was that he started to itch all over but the itching was particularly bad in the phantom leg, and he found himself trying to scratch a non-existent skin.

INGRAM Itching and pain, are they related? May we hear something about proteolytic enzymes in this regard?

WEDDELL. A barely tolerable itch can easily be evoked from the lips by stimulating them with a fine bristle mounted on a rapidly vibrating tuning fork. After one or two applications of the bristle it is possible to evoke the itch by simple contact. Further stimuli with the bristle leads to an ever increasing area surrounding the point of stimulation from which itching can be evoked by simple contact. Spreading excitation of this kind is at once removed by firmly rubbing the affected zone. Spreading inhibition, that is the temporary creation of an ever-extending zone from which itching cannot be evoked in this way can be achieved by stimulating in the same kind of way but using a low frequency tuning fork. These experiments can be carried out by oneself on oneself or upon patients. Itching of this kind can be evoked from skin anywhere over the body surface by choosing a suitable set of tuning-fork frequencies, but for demonstration purposes it is most easily evoked from the lips.

In subjects in which an antero-lateral tract on one side of the spinal cord has been severed it is no longer possible to evoke itching of this kind from the affected zone of skin, yet the bristle can be recognized and feels exactly the same as it does when it is applied on the opposite side after spreading inhibition has been built up Some observers might regard the sensation evoked in the manner which I have described as tickle but it seems to me to correspond closely to what is termed pruritus clinically

WRIGHT There is very general agreement that not only the intensity

but also the quality of a cutaneous sensation, depends to some extent on the number of receptors involved, and that a reduction in the number or an increase, may alter the quality or type of the sensation this has been argued, to relate to itching and pain. Now we know that in old age there is a gradual decline in the numbers of neurones in all parts of the body peripherally and centrally could it be that this decline accounts for alterations in the quality of some sensations? More specifically could it be that a stimulus which in a young person caused one type of sensation would, in the older person, cause itching—merely because of the fall in the number of neurones in some particular part of the nervous system?

INGRAM. I was interested that something like 25 per cent of superficial innervation could be destroyed without altering sensation. I assume that eczema, dermatitis or any inflammation of the skin would be associated with considerable destruction of superficial nerves. I think we, as clinicians, do not observe much altered sensation even with extensive dermatoses. Would Dr Weddell comment on this?

WEDDELL. I have examined hairy skin from a number of patients having a wide variety of skin affections. In many instances the affected skin contained end-bulbs together with degenerating and regenerating nerve fibres whereas skin from the unaffected zones in such patients was quite normal. In none of the cases I am referring to did the patients complain of a diminution of sensory acuity in the affected zone of skin although in certain subjects as many as one in every four of the nerves seen appeared to be morphologically abnormal. In cases in which even more nerve fibres than this were affected careful sensory tests did reveal a minimal loss of sensory acuity It might be possible to develop tests which would detect small degrees of damage but they would be bound to be elaborate, for the arrangement of the nerve fibres in the skin is such as to provide compensation against all but the most severe damage.

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HISTOCHEMICAL INVESTIGATION OF THE SKIN

THE PRINCIPLES OF HISTOCHEMISTRY

By A. G EVERSON PEARSE

πολλῶν ὀνομάτων μορφὴ μία.
AESCHYLUS, *Prometheus.*

INTRODUCTION

One shape of many names, one science of many names, such is the science of the chemistry of the tissues which forms the subject of this article. Histochemistry cytochemistry histotopochemistry histochemistry *in situ*, microscopic histochemistry physiological histology histological topochemistry microchemistry chemical microscopy analytical cytology biochemical cytology these are by no means all the names which can be found in the literature, describing essentially the same thing

It is easy to begin this introduction by defining the word histochemistry (which properly contains cytochemistry as its smaller part) as the chemistry of the tissues. Difficulties begin when we try to consider its secondary or practical meanings. This is because the word can be used to cover such widely differing methods as those of the pure protein, carbohydrate and lipid chemists on the one hand, and those of the botanist, the cytologist, and the histologist on the other Somewhere between the two come the methods of the biochemist and, sometimes, those of the pharmacologist. All those whose investigations lead them to study the nature and function of animal or vegetable tissues are histochemists in the broadest sense.

So we find, in the literature, a succession of endeavours to tie down the words histochemistry or microchemistry by the use of definitions. In his dissertation on microchemistry for instance, Grutterink (1910) defined this science as part of analytical chemistry which occupies itself in studies of chemical reactions under the microscope Later Klein in his *Praktikum der Histochemie* said that the final demand of histochemistry was for localization. It is with the expression, in succinct form, of this idea that many others have been preoccupied. Lison (1953) proposed that the qualifications *in situ* and *extra situm* should be applied in order to distinguish the two main divisions of histochemistry and Voss (1933 1952) suggested the insertion of the Greek τόπος (place or location) to make the single word histotopochemistry This would define briefly and accurately the second division of histochemistry In his text-book upon the subject Gomori (1952) incorporated the word microscopic into the title for the same purpose.

possession of the trademarks histo- and cytochemistry. At one stage there was a division between the two when attempts were made to apply the term cytochemistry exclusively to those fields now called analytical or biochemical cytology. With the healing of this division the word cytochemistry should be free to return to the fold as that brand of its parent histochemistry which deals with intracellular localization.

In all the foregoing there has been little mention of that great schism which afflicts histochemistry. This is the division, at present much more emphatic than it should be, into qualitative and quantitative branches. The larger number of qualitative techniques (some 250 or so) are the province of the applied histochemist; the smaller number of quantitative techniques remain for the most part in the hands of those who regard themselves as analytical cytologists. Some of these techniques, such as autoradiography and microradiography, did not belong to histochemistry in the first instance. Many others, however, have been removed from histochemistry by the mere application of optical techniques of mensuration. This cannot be allowed. Histochemistry suffers many other losses. To histology, for instance, goes any method demonstrating a particular morphological entity which is found to be sufficiently easy for universal application. Losses of this variety, however, can be replaced by the conversion of histological methods into histochemical ones by investigation and explanation of their mechanisms. Histology is a *forme fruste* of histochemistry since the latter deals not only with structure but with the integration of structure with function.

It is the outstanding advantage of histochemistry that it is able to demonstrate structure or function together. At the same time it provides the only way to distinguish between one cell and another in the matter of content (analytical histochemistry) or function (functional or enzymic histochemistry).

It should be axiomatic that histochemistry be not asked to do anything that can be done better by the techniques of some other discipline. Here one has in mind particularly biochemistry, whose methods and approaches serve the younger science for guidance and stimulation.

THE HISTORY OF HISTOCHEMISTRY

Origins and early history

It is generally agreed that histochemistry is as old as the science of histology but a measure of disagreement remains on the question whether the works of the nineteenth- and early twentieth-century histochemists, microchemists and physiologists, are to be regarded as part of a continuously developing

scientific discipline or as isolated and unconnected efforts. There can be little doubt that the French pharmacist, François-Vincent Raspail (1794-1878) was the first person to set down in writing (1825*a*, *b* 1829) a clear appreciation of the aims and intentions of the science of microscopic tissue chemistry. Besides being a pharmacist and inventor of a liqueur, a headache pill and a kidney powder (only the first of which survives today) Raspail was the author of numerous tracts and publications antagonistic to the medical profession. More details of his life may be found in a series of papers by Harms (1931–2) which deal with the subject in great detail and also in Baker's (1943) *Quekett Microscopical Club Monograph* on the discovery and use of colouring agents in biological microtechnique. Raspail was also a botanist of repute and his histochemical techniques, like others of the same period, were exclusively applied to botanical material.

Animal histochemistry was largely destructive in its early stages, that is to say the results of the various methods were not worth examining under the microscope. Text-books concerned with these more or less destructive methods appeared at intervals (Lehmann, 1842; Robin & Verdeil, 1853; Schlossberger, 1856), and the first to incorporate the word histochemistry into its title was Frey's *Handbuch der Histologie und Histochemie des Menschen* (1867). In 1874 Frederic Miescher produced his *Beitrag zur Histochemie* but by now the original science was beginning to split into several parts. One was to be lost for ever as biological chemistry and another became part of the science of physiology. Only a small portion remained in the hands of histologists and cytologists, and even this was to become almost completely submerged by the wave of enthusiasm which followed the application of the aniline dyes to histological techniques. During the first half of this period, which lasted from 1862, when Beneke first used an aniline dye in histology, to 1900, the science of histochemistry was kept alive by Paul Ehrlich, Frederic Miescher and by a number of authors less well known whose work continued in the tradition of Raspail. In the second half of the period of submersion, lasting from 1900 to 1936, histochemistry was maintained by workers like Gustav Mann whose *Physiological Histology* (1902) is still a source of inspiration and knowledge, and Macallum, whose Methoden und Ergebnisse der Mikrochemie (1908) became the classical text on the subject. Later in the century there was still little evidence of reviving interest in histochemistry though Parat (1927) was able to write a 'Review of Recent Developments in Histochemistry' and Patzelt (1928) a monograph on *Animale Histochemie*. A year later two histochemical works appeared which may be regarded as the forerunners of the renaissance of histochemistry. They were *Praktikum der Histochemie* by Klein and *Histochemische Methoden* by Hertwig.

The Renaissance

The new science of histochemistry without tissue destruction was proclaimed by Lison (1936) in his great work *Histochimie Animale* As I have said elsewhere, it is impossible to overestimate the effect of this book upon the progress and practice of histochemistry Without subtracting any credit from the author of this work it is possible to surmise that its publication coincided with an upsurge of interest in the possibilities of histochemical techniques, particularly on the part of cytologists and zoologists, with histologists less obviously concerned. I believe that the science which has grown up in the past twenty years as histochemistry is a direct extension of the older science created in essence by Raspail. It must be admitted that at certain periods real continuity is hard to recognize and that in others very little active histochemistry was being done. Other historians of histochemistry may disagree with this conception of a continuous science, as did Lison himself On the other hand, many will continue to doubt whether histochemistry should exist as a separate science rather than as a collection of techniques employed at times by the basic biological sciences. Until recently I took this view myself

There can be no doubt, however that histochemistry must be now recognized as a fully independent science and that histology cytology haematology embryology zoology botany pathology bacteriology physiology pharmacology and perhaps even enzymology and biochemistry by using its ever widening selection of techniques, must contribute something to its growth as well as to the furtherance of their own ends. My reasons for declaring the independence of histochemistry are many First, there has been an exceptionally rapid increase in the number of available histochemical techniques, from a hundred or so in 1945 to well over 200 today These have been produced by people calling themselves histochemists and a large number of theoretical histochemists must be at hand to interpret and explain these methods for use by workers in the basic sciences. Secondly the methods of modern histochemistry are no longer drawn straight from those of chemistry and biochemistry although these sciences continue as before to provide much of the necessary stimulus to invention. Many of the newer techniques, produced solely for histochemical use, have been adapted for use in biochemistry or in paper chromatography and paper electrophoresis. Thirdly it is no longer possible to stand out against the current view manifest in many countries by the founding of journals devoted solely to histochemistry that the infant science has at last come of age.

A CLASSIFICATION OF HISTOCHEMICAL METHODS

In a contribution to the University of London series of *Lectures on the Scientific Basis of Medicine* (vol. IV p. 358, 1954–5) I put forward the classification of the applications of histochemistry which appears below

Applications of histochemistry

QUANTITATIVE		COMPARATIVE
	ANALYTICAL	
SEMIQUANTITATIVE		MORPHOLOGICAL
	FUNCTIONAL	
QUALITATIVE		DIAGNOSTIC

The headings in the three columns appear on different lines in order to suggest that at any one time a given method may be used in a manner qualitative, analytical and comparative, or alternatively in a manner quantitative, functional and diagnostic, or in any combination of headings from the three columns, or from the first two alone. In classifying the actual methods it is necessary to use a different terminology The one I use, which is given below is partly derived from the classification given by Baker (1951) and partly from its modification by Vialli (1955). I have omitted entirely however those methods described by both these authors as methods *extra situm*.

The third column refers particularly to the final reason for using a method. Thus, comparative might mean to compare two samples of lipofuscin from different sources, with a view to determining their identity or otherwise. A morphological use might be purely that, or perhaps a use for the purpose of recording characteristics. This use of histochemistry to record the reaction of a material without drawing conclusions is often regarded as banal. On the contrary it is a most valuable one which enables workers all over the world to make quick and essential comparisons between their material and that of others The word diagnostic in this table refers mainly to problems of human pathology where, for instance, histochemical aid may be sought in the separation of tumours of the bile ducts from those of the pancreas, and in similar ways.

A classification of the in situ methods of histochemistry

PHYSICAL

I *Analytical non-destructive qualitative*

(1) Determination of refractive index (phase contrast microscopy interference microscopy).

(2) Primary Fluorescence

(*a*) Unfixed tissues

(*b*) fixed tissues.

(3) Determination of refringence (polarization microscopy)
  (a) Optical sign
  (b) degree of birefringence
  (c) dichroism.
(4) Absorption of electromagnetic rays (spectroscopy).
(5) Production of electromagnetic rays (fluorescence spectroscopy).
(6) Substitution of radioactive elements (autoradiography).
(7) Solution of non reactive dyestuffs (fat-dye methods).
(8) Resistance to solution
  (a) In native condition
  (b) after treatment.

II *Analytical, destructive qualitative*
(9) Solubility (extraction methods).
(10) Microincineration.
(11) Emission histospectrography
(12) Melting-point determination.

III *Analytical, non-destructive quantitative*
(13) Absorption of electromagnetic rays (microspectrophotometry micro-radiography microdensitometry).
(14) Autoradiography with grain-counting
(15) Measurement of mass (interference microscopy).

IV *Fractional, non-destructive qualitative*
(16) Substitution by radioactive elements (autoradiography).

CHEMICAL

V *Analytical, non-destructive, qualitative*
(17) Chemical reactions on native or fixed tissues
  ( ) Coloured end-product
  (b) colourless end product demonstrated by (1) dyeing (2) chemical reaction (3) fluorescence.
(18) Chemical reactions after treatment by
  ( ) Hydrolysis
  (b) oxidation
  (c) reduction
  (d) sulphation
  ( ) methylation
  (f) phosphorylation
  (g) acetylation
  (h) other reactions.
(19) Dye-absorption and dye-chelation (native or fixed tissues) giving
  (a) Coloured product
  (b) fluorescent product.
(20) Dye-absorption and dye-chelation (treated tissues) giving
  ( ) Coloured product
  (b) fluorescent product.

(21) Antigen-antibody reactions (immunohistology)

(*a*) Fluorescent (fluoresceinisocyanate, dimethylamino-naphthalene sulphonyl chloride, lissamine rhodamine sulphonyl chloride, etc.)

(*b*) radioactive.

(22) Abolition of pre-existing reactions.

(23) Quenching reactions (reversal of fluorescence).

VI *Analytical, destructive qualitative*

(24) Enzymal analysis.

(25) Bleaching methods (pigments).

(26) Removal by chemical reagents.

(26) Microelectrophoresis.

(28) Microchromatography

VII *Functional, non-destructive qualitative*

(29) Reaction as enzyme with selected substrates

(*a*) Coloured final reaction product

(*b*) colourless product demonstrated by (1) dyeing (2) chemical reaction (3) fluorescence (4) radioactivity

VIII. *Functional, destructive quantitative*

(30) Estimation of enzymic end products

(*a*) After conversion

(*b*) after removal.

It is very necessary before setting out to use histochemical methods for the solution of particular problems to think logically about what methods are available and what form of information each can be expected to give. Reference to a comprehensive classification, such as that given above, is probably the most convenient way to begin. A great deal of applied histochemistry some of which is nevertheless of great value, seems to be carried out without its authors having a clear notion of their reasons for using the methods they have chosen and in the apparent ignorance of applicable methods which have not been used. In many cases the reason for not using a method is that it cannot be made to work. This is a good reason and it should be stated rather than concealed.

It is of course very necessary in histochemistry to use, if possible, every reaction which may give evidence on the nature or function of a particular structure. Few methods have such high specificity not to mention sensitivity that they can be relied on singly One accepted exception that comes to mind is the Feulgen nucleal reaction for DNA but others are hard to find.

Many of the headings in the above classification of histochemistry are self-explanatory but others may be obscure even to those familiar with the techniques in question. Until recently refractive index determinations (I 1) were carried out by using phase contrast microscopy in conjunction

with different liquids having indices the same as that of the object in question. They can be carried out with greater convenience by means of the interferometer microscope. The determination of primary fluorescence (I, 2), *the emission of light on visible wave-length upon excitation by* ultra violet light (300–400 mμ), is an old histochemical method, derived *directly from the original researches of Köhler (1940a, b) on the ultra* violet microscope. It is important to realize that fixation, especially with formalin, alters *primary fluorescence in many ways. It may quench some* types of fluorescence altogether and alter the wave-length of emitted light in other cases. By combining with diffusible molecules such as nor adrenalin and 5-hydroxytryptamine to form new fluorescent compounds formalin produces what should strictly be called secondary fluorescence. This term (19*b* 20*b*), however is reserved for fluorescence induced by dyes which are usually fluorescent themselves, that is fluorochromes.

Determination of refringence (I, 3) has played only a small part in histochemistry up to the present time, being restricted largely to the study of substances in crystalline form such as uric acid, calcium phosphate, the digitalin-cholesterol complex and various lipids. Distinction between positive and negative birefringence has seldom been attempted, and similarly little attention has been paid to the question of form and intrinsic birefringence. With the advent in recent years of microscopes able to detect very weak birefringence in histological materials cytologists have made considerable advances in studying structures like the mitotic spindle, but these advances have not been reflected in applied histochemistry

The measurement of the absorption of electromagnetic rays (I, 4 III 13) is the basis of most of the quantitative methods of histochemistry Staining methods *per se* depend on the absorption of light of different wave lengths and some staining methods, as well as methods resulting in the deposition of dyes, have been used as a basis for microspectrophotometry This has also been called histophotometry and cytophotometry The two main divisions *of microspectrophotometry are (1) visible light, and (2) ultra* violet light, and for the former two histochemical reactions have been extensively used. These are the Feulgen nucleal reaction and the *Millon* reaction for tyrosine. Methyl green, fast green and naphthol yellow are dyes which have found favour for studies of this kind.

Commercially produced microspectrophotometric instruments are now available in several countries, most of them based on RCA photomultiplier tubes of one sort or another In conjunction with a set of filters these can be used to measure the absorption of visible light within selected narrow wave-lengths. With the addition of a monochromator the absorption curves of cells can be determined. It is to be expected that in the near future most

histochemical laboratories will be equipped for microspectrophotometry in visible light and that comparative measures of the kind which play so large a part in histochemical researches will be made by this means. Ultra violet microspectrophotometry because of the greater complexity of apparatus required, is unlikely to be much used in applied histochemistry but increasing use of Coons's fluorescent antibody technique (V 21), now incorporated as the new science of immunohistology will make some form of fluorescence spectroscopy (I 5) essential. Difficulties in this case are mainly connected with the low intensity of the emitted light and these have already been overcome in certain laboratories.

An important limitation in many fields of histochemistry is the impossibility of obtaining *absolute* quantitation. This is regarded by some authorities as the Achilles heel of the whole science. I am not so sure that it is so. *It is often possible to achieve relative quantitation of a method with little trouble, and little extra information is to be gained from the possession of figures for absolute amounts of substances or enzyme activities, except in special cases.*

Little comment is necessary in the case of section II of the classification except to say that little use has been made either of emission histospectrography (II 2), or of melting-point determinations (II 12). In section III quantitative autoradiography (III 14), and dry mass measurement (III, 15) both represent techniques of which more will be heard in the future. The former can be carried out by densitometry or by grain-counting which is probably more reliable. Grain-counting or densitometric measurements can be used for quantitative studies of enzyme activity in cells (VII 29) after methods of conversion of the end-product (VIII 30) have been applied. Dry mass measurements with the interference microscope are based on the fact that the dry mass per unit area is proportional to the difference between the optical path of the cell and the optical path of an equal thickness of water The proportionality factor (I/χ) is about 0·19 *for* proteins 0·17 for lipoproteins and from 0·16 to 0·20 for the nucleic acids. A mean value *of* 0·18 *is usually selected in making dry mass measurements.*

On the whole, the chemical *methods in the classification* are self *explanatory The reactions listed in section (V 18) have been increased* enormously in the past few years and they include a number of so-called blocking reactions which are of considerable importance in modern histochemistry

Very notable advances have been made in histochemical antigen-antibody reactions through the work of Coons and his collaborators and, subsequently an increasing number of investigators. Most of these have used the original technique of coupling fluoresceinisocyanate to the protein and viewing the final result by fluorescence microscopy *Recently*

other dyes have been used which are easier to synthesize than fluorescein-isocyanate and which couple with proteins in aqueous solutions, so that denaturation and loss of reactivity is avoided. In the near future the techniques of immunohistology may well become routine diagnostic techniques in histopathology. As an alternative, radioactive compounds such as ^{131}I have been used for labelling protein antibodies followed by autoradiographic methods of localization. These have not been so popular as the fluorescence methods.

Some advances have been made in the methods of enzymal analysis (VI, 24) chiefly by the use of the purest samples of enzyme which are now commercially available. Unfortunately, as with all the destructive methods of analysis, specific removal of one substance may lead, non-specifically, to the removal of others. The two techniques (VI, 27, 28) of micro-electrophoresis and microchromatography are in a very early stage of development and it is as yet too early to say whether, when the real technical difficulties have been overcome, useful and practical methods will be evolved. Certainly it should be possible to identify diffusible substances of small molecular weight by recording their movement in tissue sections.

In no section of histochemistry have more striking advances been made in the past two years than in the field of enzyme reactions (VII, 29). Methods are now available for more than forty five enzymes and, in the applied field, the number of papers based on enzyme reactions at least equals the number in all other fields put together. This emphasis on the functional side of histochemistry is a pointer to future advances. The application of quantitative measurements to enzyme histochemistry, either by spectrophotometry (III, 13) or by techniques such as those recorded in VIII, 30 will, I consider, raise this part of the subject to a position of pre-eminence before many years have elapsed. The main advances of recent years have been in the field of dehydrogenase histochemistry and it is possible today to localize with considerable accuracy nine of the specific dehydrogenases, together with succinic dehydrogenase and the two co-enzyme linked diaphorases (DPNH-diaphorase, TPNH-diaphorase). These last two flavoprotein enzymes transfer hydrogen from the reduced DPN or TPN acceptor dyes. As far as histochemistry is concerned these are always of the tetrazolium class. While the DPNH-diaphorase and succinic dehydrogenase methods may be used in conjunction to demonstrate the distribution of mitochondria in cells of every type, the methods for the individual specific dehydrogenases permit one to distinguish minor changes in the oxidative pathways of the cell. Moreover, biochemical knowledge of the function of the dehydrogenases and of the mechanisms by which they act is much more precise than it is in the case of most other enzyme

systems. We are, therefore, in a position, as never before, to reveal something of the mechanisms by which the cell carries out its functions in health and disease and to begin to describe the organization of events within the cell.

Modern histochemical dehydrogenase methods not only afford excellent (intramitochondrial) localization but also allow one to assess the physical state of the mitochondrial membrane as far as its permeability is concerned. Very early damage to mitochondria can easily be detected and we can thus distinguish not only differences in functional status between one tissue and another as can the biochemist, but between one cell and another and between one mitochondrion and another. Results of this order cannot be achieved by the techniques of any other scientific discipline.

It is impossible to pick up a copy of any journal of pathology, not to speak of journals of anatomy, biology and zoology, without finding a dozen examples of investigations where histochemical techniques could be applied simply and with obvious advantage.

Histochemistry continues to go forward, rapidly, on a number of narrow fronts. What is of far greater importance to my way of thinking is the steady advance of *applied histochemistry*, the volume of which is increasing every year in most branches of the basic sciences to which histochemical techniques are applicable. Nous avouons, says Lison, que nous-même avons subi des crises de pessimisme about histochemistry. For my part, although from time to time I have doubts about the specificity or sensitivity or general utility of many of the methods it employs, for the whole science of histochemistry and for its future I have no doubts at all.

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ESTERASES IN NORMAL HUMAN SKIN AND IN CHRONIC GRANULOMATA

By G C. WELLS

For alkaline phosphatases, for acid phosphatases and for simple (non-specific) esterases we have histochemical methods which have been widely used in the last few years, and the details of these methods are to be found in standard works on histochemistry (Gomori, 1952 Pearse, 1953 Lillie, 1957) What I want to do is map out the sites where these enzyme systems are demonstrable in high concentration in (1) normal human skin, and (2) chronic cutaneous granulomata.

In this account I have drawn upon the work of others as well as upon work of my own. In attempting to give a composite picture of enzyme localization some compromise has had to be reached over differences between workers. These differences inevitably arise when varying details of tissue preparation are used and when observations are related more to work with one substrate than with another My aim has been to show the sites of intense hydrolysis, and with this I have tended to ignore sites of dubious or slight enzyme activity Nachlas, Young & Seligman (1957) have pointed out that false negative errors are just as erroneous as false positive ones, so in this account, allowance must be made for under-estimation of doubtful enzyme sites.

A map of enzyme localization in skin is necessarily an over-simplification, since it makes no allowance for functional changes during cyclical activity as, for example, in hair growth or in sweat secretion (Montagna, 1956).

ALKALINE PHOSPHATASE

The calcium phosphate precipitation method of Gomori (1939) and Takamatsu (1939) for demonstrating alkaline phosphatase in tissue sections, has been widely used in qualitative histochemistry

Recent developments in quantitation which employ analytic chemistry and interference microscopy bear witness to the usefulness of the calcium phosphate precipitation method (Danielli 1958). Danielli indicates that with *unfixed* frozen sections, release and capture of phosphate ions can be accurately related to enzyme activity Sites of weak or doubtful precipitation can be evaluated after application of a microscopic beam of ultra-violet light, which destroys true alkaline phosphatase activity but does not affect non-enzymatic precipitation. While frequent reference to unfixed material

is a necessary check, comparable but weaker qualitative results can be got after fixation in cold formolsaline for up to 24 hr before making frozen sections, and a good deal of work on the skin has been done in this way. An alternative histochemical method for alkaline phosphatase (Gomori, 1951) using substrate of α naphthyl phosphate from which naphthol is released by enzyme action and coupled with a diazonium salt, does in fact give results comparable with the calcium phosphate method and at the same time avoids certain artifacts.

In working with skin it is particularly important to have adequate controls through incubating sections from the same block with all the ingredients of the incubating medium except the glycerophosphate. Specific inhibitors such as cyanide or cysteine also provide an important check on results, and dependence of the enzyme on Mg ions can be shown. In skin, sources of error with the calcium phosphate precipitation method are

(1) Confusion of cobalt sulphide with melanin or other dark pigment in the skin.

(2) Affinity of inorganic ions for certain areas of the surface keratin and for inner root sheath keratin.

(3) Calcification. That is, if calcium phosphate is already present in the skin the cobalt sulphide reaction will be positive.

(4) False localization. Under some conditions there may be a shift of enzyme or its reaction products which are then taken up at areas of special affinity for example, in nuclei or in the granular layer of the epidermis.

The alternative method with naphthyl phosphate avoids most of these difficulties. Useful accounts of the distribution of alkaline phosphatase in normal human skin and in some pathological conditions have been given by Fisher & Glick (1947), Pirila & Eränkö (1950), Spier & Martin (1956) and by Kopf (1957).

ALKALINE PHOSPHATASE IN NORMAL SKIN

Epidermis

No reaction for alkaline phosphatase is detectable in normal human epidermis. With the calcium phosphate method, apparent staining in the granular layer has caused difficulty and some observers have considered this to be a positive reaction. Nadel & Wodinsky (1955) studied this point and concluded that the keratohyalin granules had an affinity for alkaline phosphatase and for calcium phosphate and that staining at this site was a diffusion artifact. With the naphthyl phosphate method, the granular layer shows no staining. In some acanthotic conditions Kopf (1957) reports alkaline phosphatase in the thickened granular layer.

Pilosebaceous unit

The epithelial structures of the hair follicle show no reaction for alkaline phosphatase, though care must be taken to discount false-positive reaction sites from non-enzymatic ion binding, as seen in the inner root sheath of the hair follicle. The connective tissue and vasculature of the hair papilla and of the immediate surround of the hair bulb show very strong alkaline phosphatase reaction. Alkaline phosphatase disappears from this region when the hair stops growing and when the hair sheath starts to involute, but it is present in the empty resting follicle (Kopf & Orentreich, 1957).

Sebaceous gland

The sebaceous gland is devoid of histochemically demonstrable alkaline phosphatase, but the gland is surrounded by a network of capillaries which show up well by virtue of their alkaline phosphatase content.

The eccrine sweat gland unit

Some cells of the sweat gland epithelium show strong alkaline phosphatase activity in their cytoplasm and an appearance of positive staining is described in the walls of the intercellular canaliculi (Montagna, 1956). The myoepithelial cells are strongly positive. The sweat duct appears to be devoid of this enzyme.

The apocrine gland

The tall columnar cells of the apocrine gland show alkaline phosphatase activity fairly strongly and it seems to be arranged in a bipolar fashion, at the base of the cell and at its luminal border. The myoepithelial cells are strongly positive.

The blood vessels

The linings of the capillaries show intense reaction for alkaline phosphatase, and this is to be seen throughout the dermis. Plate 1 fig. 1 shows an unfixed section of human skin cut on the Pearse cold microtome and incubated in the calcium-glycerophosphate substrate. The capillary walls are seen to be densely blackened by the deposition of cobalt sulphide at the site of alkaline phosphatase. The rest of the skin in this section is seen to be devoid of alkaline phosphatase activity.

Chronic cutaneous granuloma

Alkaline phosphatase was not demonstrable in the substance of most of the granulomata studied: that is, the giant cells, macrophages, epithelioid cells, plasma cells and lymphocytes appeared to be negative. Capillary walls were positive, and the degree of vascularity of the granuloma obviously influenced

the alkaline phosphatase staining. For example, a young lupus nodule is seen to be devoid of alkaline phosphatase, and the avascular knot stands out from the rest of the dermis with its positive-staining capillaries. On *the other hand, a rather vascular granuloma such as leishmania cutis showed* the positive capillaries in amongst the inflammatory cells. Furthermore, healing alters the picture at the periphery of a granuloma. Not only are the capillaries alkaline phosphatase positive, but also the young connective tissue fibres and fibrocytes. In some cases lymphocytes at the periphery of a lupus nodule are said to be positive (Klingmüller 1953 Kopf 1957)

Text fig 1 shows a line drawing of the skin and its appendages upon which the areas of high alkaline phosphatase activity are marked in blue. The left centre vertical panel shows cellular details of a chronic granuloma. The right centre panel shows enlargement of the epidermis and detail of eccrine and apocrine sweat apparatus.

Comment

In spite of the large amount of work that has been done on alkaline phosphatase throughout the body we know nothing of its function in skin. It seems to be present where active repair is going on, and it is found in the stroma of the hair papilla when hair growth is in progress, or in anticipation of growth. The delineation of capillary walls is so good with methods for showing alkaline phosphatase that the technique has recently come to be used in an anatomical sense. Ellis, Montagna & Fanger (1958) have used thick sections and a method for alkaline phosphatase to describe the blood vessels supplying the appendages of the skin. Klingmüller (1958) has employed much the same technique together with stereoscopic photographs to reconstruct the blood supply to normal and pathological skin.

ACID PHOSPHATASE

When a histochemical method for demonstrating acid phosphatase was first introduced (Gomori, 1941), none could be shown in skin (Gomori, 1941 Fisher & Glick, 1947 and Pirilä & Eränko, 1950). The original lead phosphate method was capricious, and fixation and embedding processes inhibited the enzyme. With fixation in cold calcium formalin followed by frozen sectioning, and with optimal substrate concentration, the method can still be used with advantage (Gomori 1956). The α naphthyl phosphate azo-coupling method of Seligman & Manheimer (1949) gives a vague idea of acid phosphatase distribution in tissue sections, but the azo-coupling at pH 5 is slow and localization of dye is very poor The newer post coupling method of Rutenburg & Seligman (1954) gives better results.

Here 6-benzoyl-2 naphthyl phosphate is first hydrolized, and then as a second step at more alkaline pH azo-coupling is effected. Recent reports (Spier & Martin, 1956 Moretti & Mescon, 1956) give accounts of the distribution of acid phosphatase in normal human skin, and in the course of their work Moretti & Mescon compare results by all three methods, and with different preparatory procedures.

A disadvantage of all the methods for acid phosphatase is the increased deposition of precipitate or dye with time of incubation, and the end-point for describing positive sites of activity is arbitrary It seems reasonable at present to take a point at which the granular layer of the epidermis shows strong cytoplasmic staining, before there is any general nuclear staining in the epidermis, and in relation to this, other sites of activity may be judged. A distinctive characteristic of acid phosphatase is the clear-cut inhibition produced by sodium fluoride.

I have recently found the lead phosphate precipitation method for acid phosphatase much more useful since Dr S J Holt pointed out to me that the substrate should be freshly prepared every few days, and not stored in the refrigerator for several months as Gomori (1952) originally did. This method, following calcium-formalin fixation in the refrigerator and frozen sectioning has given good results with short incubation ($\frac{1}{2}$–2 hr at 37 C.). Clear-cut results have recently been obtained with various chronic granulomata of skin.

ACID PHOSPHATASE IN NORMAL SKIN

The epidermis

With histochemical methods for acid phosphatase there is general epithelial staining which increases with time of incubation. The staining appears to be predominately cytoplasmic. It is most intense in the granular layer and shades off towards the basal layer of the epidermis. The surface keratin shows no reaction.

Pilo-sebaceous unit

The bulb of the hair follicle is strongly positive and the keratogenous zone of the hair shaft shows intense acid phosphatase activity The mature cortex of the hair shaft is negative, though medulla (if present) appears positive The root sheaths are moderately positive.

The sebaceous gland shows moderate acid phosphatase activity in its periphery (germinal epithelium) and fairly strong positive staining of walls of developing sebaceous cells. The new formed sebum is negative, but there is a very intense acid phosphatase staining of the walls of the sebaceous canal.

The eccrine sweat gland unit

The sweat gland acinar cells show minimal staining for acid phosphatase, and such as can be seen is at the luminal margins of the secreting cells. The sweat duct shows slight acid phosphatase reaction diffusely through out the cuboidal cells of its wall. The intra-epidermal portion of the sweat duct is cuffed by fairly intense acid phosphatase reaction which corresponds with the presence of a granular layer in this part of the duct wall.

The apocrine sweat gland unit

There is some acid phosphatase reaction in the epithelium of the apocrine gland and it is mainly concentrated at the luminal borders of the columnar secreting cells. The duct shows very faint staining, apart from variable luminal content, and the myo-epithelial cells appear negative.

Acid phosphatase in chronic granuloma of skin

With improved technique, the intense acid phosphatase staining of histiocytes (macrophages) and giant cells is well seen in the chronic cutaneous granulomata, whether infective or not. If incubation is short (about 1 hr) only the granular layer of the epidermis may be strongly stained, and at this point the only positive staining elements of the granuloma are these histiocytes. If incubation is continued longer lymphocytes and plasma cells become positive, and widespread nuclear staining may ensue.

Pl. 1 fig. 2 shows a calcium-formalin fixed frozen section of a peri follicular granuloma (oil acne) incubated for 1 hr in the Gomori substrate. Areas of strong acid phosphatase reaction show black. The granular layer shows intense acid phosphatase activity but the rest of the epidermis shows little. In the infiltrate there are numerous macrophages which show up black by reason of their acid phosphatase content. At this stage the other cells of the infiltrate and the rest of the dermis are negative for acid phosphatase.

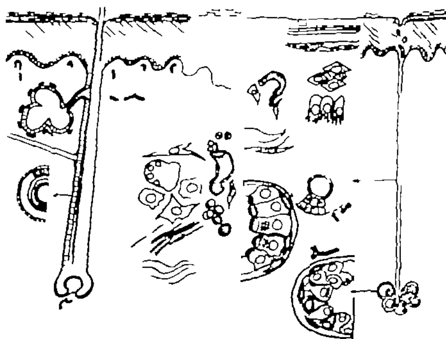
Over the same line drawing as was used in Text fig 1 acid phosphatase concentrations are marked in green (Text fig 2).

Comment

Concentration of acid phosphatase in the keratogenous zones of surface epithelium and of hair and in the sebaceous gland and duct has led to the suggestion that at these sites the enzyme is concerned with phospholipid metabolism. This is, however no more than speculation since the natural substrates for acid phosphatases in skin are not known.

Key to Text-figures -3

The following text-figures are divided into four vertical panels. The left-hand panel contains a diagram of normal skin with hair follicle and sebaceous gland. The right-hand panel shows an eccrine sweat gland and duct. The right central panel shows enlargement of normal structures (epidermal cells, dermal papilla, connective tissue, and both kinds of sweat gland). The apocrine gland is shown on the left on th panel. The left central panel shows details of pathological changes (acanthosis and parakeratosis and the giant cells, histiocytes, leucocytes, young connective tissue elements and capillaries found in the chronic granulomata).



Text-fig. Alkaline phosphatase (blue).

Text-fig. Acid phosphatase (green).

Text-fig. 3. Simple esterase (red)

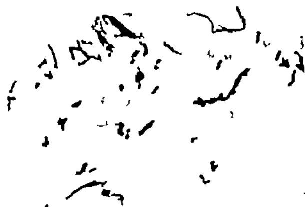
In the chronic inflammatory infiltrates there is this striking concentration of acid phosphatase in histiocytes or macrophages and giant cells. This observation is in line with reports on these cells in various species, as for example that of Grogg & Pearse (1952) showing strong acid phosphatase in the phagocytes of certain animals, especially in experimental tuberculosis. Osteoclasts of bone show strong acid phosphatase activity (Schajowicz & Cabrini, 1958). The chicken monocytes in tissue culture develop as macrophages in certain circumstances and then show acid phosphatase activity (Weiss & Fawcett, 1953). And Doyle (1955) finds this type of cell in the rabbit's meso-appendix to be rich in acid phosphatase as well as peptidase. Recently Braunstein, Freiman & Gall (1958) have shown acid phosphatase and simple esterase activity in the histiocytes of lymph nodes both normal and hyperplastic, using techniques similar to those which I have applied to skin.

Simple (non specific) esterases

Since the introduction of azo-coupling histochemical techniques (Menten, Junge & Green, 1944) it has been possible to demonstrate non-specific ali-esterases in many tissues, including skin. Another principle uses substituted indoxyl esters (Holt & Withers, 1952) from which insoluble indigoid dyes are formed at the sites of hydrolysis. These simple esterases may be distinguished from specific cholinesterase and from pseudo-cholinesterase by the inhibitory effect of eserine and lysergic acid respectively. Simple esterases hydrolyse short chain carboxylic acid esters (Findlay 1955) and appear to be distinct from lipases, since hydrolysis of longer chain ali-esterases could not be shown. Histochemical methods for true lipases have not been satisfactory and I have not been able to make any reliable observations on skin with Gomori's (1952) Tween methods.

SIMPLE (NON SPECIFIC) ESTERASES IN NORMAL SKIN

In normal human epidermis some diffuse cytoplasmic staining from simple esterase activity can often be seen, depending upon the method used and upon the length of time of incubation. Most observers (Findlay 1955 Montagna, 1955 Steigleder & Schultis 1957) describe strong esterase activity in a well-defined band between the stratum granulosum and the stratum disjunctum of the keratin, that is, where the stratum lucidum or stratum compactum is to be found. This esterase-positive band has been absent from some of my sections from a variety of human skin, both normal and pathological, using naphthol A.S acetate, and 5 bromoindoxyl acetate substrates, and it is notably absent from thick epidermis of palm or sole. In some preparations particularly in those from the face, there are patches


1


2

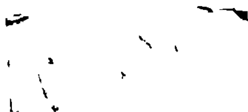


PLATE 2



Fig. 1



Fig

For explanation, see p. 34

of strong esterase staining in the more superficial parts of the horny layer and in some cases this may be related to openings of sebaceous glands. It seems as though esterase has been carried on to the surface with the out flow of sebum or possibly sweat. In specimens showing mild disturbance of the skin surface (chronic eczema) parakeratotic cells (Pl. 1 fig 3) may show strong esterase activity (Braun-Falco 1956), though this is not a consistent finding for all parakeratosis. Steigleder (1958) has made a special study of the surface esterases of the scalp and mentions that possible sources of simple esterase are sebum, sweat, keratinizing epithelial cells, and bacteria or fungi on the surface. He notes that the esterase which appears at the mouths of the sebaceous canals is peculiarly resistant to inhibitors an observation which I can confirm. Steigleder has also shown that simple esterases can be demonstrated in skin surface wipings made with filter paper

Pilo-sebaceous unit

There is intense esterase staining of the lining of the sebaceous canal, and the esterase activity can often be traced up round the hair shaft to the surface. The maturing sebum is devoid of esterase activity though with prolonged incubation some crystalline deposits of dye can be seen in the fat, but this is a false localization. The periphery of the sebaceous gland (germinal epithelium) shows slight esterase activity

The outer and inner root sheaths of the hair follicle show mild esterase reaction, and the keratogenous zone of the hair shaft and the keratinizing zone of the inner root sheath may stain, though this is variable (Braun Falco, 1958).

The eccrine sweat gland unit

In the sweat gland esterase activity is moderate but variable. Sometimes it can be seen that certain cells of an acinus have very strongly positive cytoplasm, while adjacent cells are negative (Pl. 2, fig. 1), and often whole acini appear devoid of activity The duct shows weak esterase activity of lining cells, but fairly strong staining of the lining cuticle which appears to become stronger as the duct nears the skin surface. The intraepidermal portion of the sweat duct may be lined with strongly positive material, which sometimes appears to flow out on to the keratin around the sweat pore.

The apocrine gland

The secreting cells of the apocrine glands show marked esterase activity which becomes stronger towards the luminal border of the acinar cell.

The connective tissue

Scattered throughout the connective tissue of the skin are elongated or stellate cells (Findlay 1955) which show moderately strong esterase reaction in their cytoplasm. These cells are most numerous in the pars papillaris of the dermis, though present (Pl. 1 fig 3) throughout the rest of the normal skin. Apart from these cells, the connective tissue shows no esterase activity

Simple esterases in chronic granuloma of skin

In a large number of biopsies which I have examined, I have been able to find a consistent pattern of simple esterase location. The material consists of biopsies from chronic infective granulomata such as lupus vulgaris, leprosy leishmania cutis and from granulomata in which infection is unlikely to be present such as sarcoidosis, granuloma annulare and foreign body reactions. The strongly positive cells belong to the histiocyte series and are probably macrophages These show up in the chronic granuloma as large cells, either oval stellate, or fusiform, often with long straggling processes, and the esterase staining is cytoplasmic. Giant cells whether of Langhans or of foreign body type are usually strongly positive. With the acetates of naphthol AS and of 5-bromoindoxyl epithelioid cells show variable staining, though with α naphthol acetate they appear positive (Steigleder 1957). Other components of the granuloma are negative for non-specific esterase (plasma cells, lymphocytes, capillaries) apart from positive stellate connective tissue cells already mentioned in normal skin. Pl. 2, fig 2 shows intense esterase activity confined to the cytoplasm of large cells (phagocytes) grouped round the neck of a damaged hair follicle The biopsy was made from the arm of a man who had oil acne The dark colour of these cells in the photograph is due to indigoid released from 5 bromoindoxyl acetate by enzyme action.

In some of the infective conditions it has been possible to relate strong esterase action in large histiocytes to the presence of organisms. In lepromata, the lepra cells give intense esterase activity and the globi of chronic lepromata containing mycobacterial debris have esterase positive walls (Wells, 1957) and in chronic leishmania cutis, cells containing Leishman-Donovan bodies are esterase positive. Nonetheless there are plenty of other esterase positive histiocytes devoid of organisms both in these conditions and in non infective ones such as granuloma annulare.

Using the same line drawing as in Text-fig 1 areas of intense non-specific esterase activity are shown in red (Text fig 3).

Comment

In the absence of any knowledge of the natural substrates hydrolysed by simple esterases, we can say nothing of their true mode of action or significance. That more than one kind of hydrolytic agent is present in skin is suggested by the striking resistance to inhibitors of the esterase of the sebaceous canal, as compared with the esterases of histiocytes. The approach to the problem at present remains topographical in the sense that we can demonstrate hydrolysis of simple esters in certain parts of the skin, and that is all. In the chronic granulomata the concentration of esterase is remarkable and can clearly be related to the histiocytes and giant cells. These same cells have been shown to be rich in acid phosphatase. Pepler & Pearse (1957) have pointed out that some hydrolysis of esters may be attributable to proteolytic enzymes, and peptidase activity has been demonstrated in macrophages (Burstone & Folk, 1956). However Holt (1958) showed that only a small fraction of the esterase activity of liver could be achieved by chymotrypsin, so that it is unlikely that all non-specific esterase hydrolysis demonstrable by histochemical techniques is in fact proteolytic. Evidently the macrophages that appear in these chronic inflammatory lesions of skin are well endowed with enzymes which need to be further characterised.

SUMMARY

(1) *In normal skin*

(*a*) Alkaline phosphatase is demonstrable in the capillary walls, in the hair papillae, and in the acinar and myo-epithelial cells of eccrine and apocrine sweat glands.

(*b*) Acid phosphatase is found in keratogenous zones of epidermis and of hair cortex, in the hair follicle, and in the sebaceous cells and duct. There is a small amount at the luminal borders of the eccrine and apocrine sweat glands.

(*c*) Simple (non-specific) esterase is to be found at various levels of the epidermis. It is strongly positive in the sebaceous canal, and some is to be found in the hair root. Certain cells of the sweat gland acinus are positive, and the luminal border of the sweat duct is increasingly positive towards the surface. The apocrine gland cells are positive, most strongly at their luminal borders. Scattered cells of the dermal connective tissue are positive.

(2) *In chronic granulomata of human skin*

(*a*) Alkaline phosphatase is present in the capillary walls, to an extent which depends on the vascularity of the granuloma. If repair is in progress,

alkaline phosphatase is demonstrable in young collagen fibres and in fibrocytes.

(*b*) Acid phosphatase and simple (non-specific) esterases are found in cells of the histiocyte series which contribute to the granuloma (macrophages, epithelioid cells and giant cells).

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Unfixed section of human skin cut on the Pearse cold microtome. Calcium phosphate precipitation method for alkaline phosphatase. Capillary walls are strongly positive

Fig. 2. Cold calcium-formalin fixed frozen section of human skin showing perifollicular granuloma (oil acne). Gomori lead phosphate precipitation method for acid phosphatase. Cells of the granular layer are strongly positive, and macrophages of the dermal infiltrate appear black, indicating strong reaction for acid phosphatase.

Fig. 3. Cold formalin fixed frozen section of human skin (chronic eczema). *O*-acetyl-5-bromo indoxyl method for simple esterase. Parakeratotic band in the epidermis is positive. Dermal histiocytes are positive, especially in the papillae.

PLATE 2

Fig. 1. Cold formalin fixed ester-wax embedded section of human skin (sweat gland). Naphthol A.S. acetate method. Certain of the acinar cells appear dark, and are positive for simple esterase.

Fig. Cold calcium-formalin fixed frozen section of human skin showing perifollicular granuloma (oil acne). *O*-acetyl-5-bromo indoxyl method. Large phagocytes appear black and are strongly positive for simple esterase.

THE HISTOCHEMISTRY OF KERATINIZATION

BY A. JARRETT

Keratin is the natural end product of epidermal cells it is not a single chemical compound, but shows great variations in composition. There are obvious physical differences between such keratins as hair and nail and even keratin from a given source is not of constant composition (Rothman 1954) The chemical constitution depends upon certain factors such as the rate of epidermal proliferation, mechanical friction and irradiation that are present at the time of keratinization. The underlying dermis probably also has an effect on the nature of the keratin. The study of the dynamics of keratinization is therefore complicated and requires the examination not only of the keratin itself, but of the cells undergoing keratinization and of the underlying dermis.

Keratins are composed of polypeptide chains held together by three types of cross-linkages. By far the most important of these is the disulphide linkage as this is responsible for the main strength of the keratin molecule. The mode of formation of this linkage is the oxidation of two sulphydryl groups belonging to two adjacent cysteine residues, and this results in the joining together of the two sulphur atoms with the elimination of the two hydrogen atoms as water This type of bond, together with associated high sulphur content, is the characteristic feature of keratins. The other bonds occurring in keratins are the salt linkages and hydrogen bonds. The former are ionic attractions between a carboxyl group in one polypeptide chain and an amino group in the opposite polypeptide chain. Hydrogen bonds are links between two dipoles, one of which is a hydrogen atom, the other being either an atom of oxygen or nitrogen.

THE GRANULAR LAYER

It is in the region of the granular layer that the alteration of epidermal cells to keratin occurs, and therefore this has been our site of interest in the study of epidermal keratinization. At this level the nuclei of the epidermal cells are disrupted, and the cells contain the so-called keratohyalin granules. Previous workers, Smith & Parkhurst (1949) and Leuchtenberger & Lund (1951), reported that these granules were removed by ribonuclease how ever Lansing & Opdyke (1950) were unable to confirm these findings. We

ourselves were unable to remove these granules by ribonuclease digestion, but they were removed by lipase.

Baker's acid haematin method for phospholipids clearly demonstrated the presence of these compounds in the region of the granular layer these were usually in a continuous band, but occasionally they were distributed as granules.

Oxidases in the granular layer

Incubation of alcohol fixed paraffin sections at 60 C. for 6 hr in a 1 in 500 aqueous solution of dopa showed the presence of a dopa-oxidase *in the granules themselves* This is probably a non-enzymatic oxidase as the treatment to which the sections had been subjected had destroyed the dopa-oxidases in the melanocytes.

Phosphatases in the granular layer

We have demonstrated the presence of acid phosphatases in the kerato-hyalin granules. Kopf (1957) obtained positive results for alkaline phosphatases *in the granular layer but he thought this to be an artifact due to* the selective absorption of cobalt. We agree that false positives occur in this region, and in inner root sheath and parakeratotic keratin. This is probably due to the organic phosphorus containing compounds already present in these areas, the cobalt combining with these and giving a false positive reaction.

Meyer & Weinmann (1957) reported the presence of phosphamidases in this region, but their technique was probably not demonstrating phosphamidase activity as they claim. Nevertheless, it is fairly certain that they were detecting acid phosphatases in the same situation as we found them.

Phospholipids in the keratin layer

Snider Gottschalk & Rothman (1949) described the breakdown of phospholipids during the process of keratinization, and we have shown that the complex phospholipids in normal epidermis disappear in the upper keratin layers. It is, therefore possible that the phosphatases present in the granular region are responsible for the chemical dissociation of these compounds. The breakdown of these high energy phosphorous compounds could supply the energy required for the oxidation of the —SH groups, and also for the polymerization of keratin precursors. The oxidases as detected by the modified dopa technique could also play their part in the oxidation of the —SH groups.

These views are supported by the study of parakeratotic keratin. In this mode of keratinization there is no granular layer and consequently no



For explanation see p. 40

oxidases or phosphatases have been detected. The phospholipids are therefore not removed, and the whole thickness or the parakeratotic keratin gives a positive reaction with Baker's acid haematin method. Because these lipids are not broken down less energy is available for the oxidation of sulphydryl groups, and for the polymerization of the keratin molecule. This view is supported by the findings of other workers (van Scott & Flesch, 1954 and Magnus, 1956) in that they have reported a high —SH content in psoriatic keratin. This keratin is also less compact than normal epidermal keratin, and this may be due to a lesser degree of polymerization.

This relationship between the phospholipid content of keratin, and the degree of phosphatase activity in the granular layer is beautifully shown in normal rat tail skin. In rats tails only the openings of the hair follicles have an active granular layer in the intervening regions no definite granular layer can be detected. With a modified Baker's technique that removed the contaminating phospholipids of sebum we have clearly shown that, in the region of the follicular openings where there is a granular layer with positive phosphatase activity no phospholipids are present in the keratin, whereas in the areas without a granular layer these lipids are present throughout the whole thickness of the keratin.

Inner root sheath keratin and pressure keratinization

In sections of human skin fluorochromed with thioflavine T hair and epidermal keratin fluoresce blue, whilst inner root sheath keratin fluoresces a brilliant yellow The nuclei of the cells also fluoresces a bright yellow because of this similarity it was thought possible that inner root sheath keratin might contain nucleic acids that are absent in normal epidermal and hair keratins.

Digestion with ribonuclease prior to fluorochroming with thioflavine T caused the inner root sheath keratin to fluoresce blue like epidermal keratin, whereas deoxyribonuclease digestion did not alter the yellow fluorescence of this keratin.

After ribonuclease digestion of this keratin there was a gross loss of birefringence compared with untreated inner root sheaths this is indicative that the nuclear protein is an integral part of the keratin molecule, and its removal results in the disorientation of the molecular structure.

This has been further investigated by studies with peracetic acid oxidation, and we have been able to produce evidence that the ribonuclear protein is bound to the disulphide linkages of the keratin molecule (Jarrett, 1958)

Unlike normal epidermal keratin, inner root sheath keratin contains phospholipids as shown by Baker's method. This is to be expected as the

keratin is formed without a granular layer and therefore in this respect resembles parakeratotic keratin.

Keratinization of the deeper epidermal cells has been observed in conditions of benign epidermal hyperplasia. The cells most deeply penetrating the dermis are chiefly affected. It occurs very frequently in warts, but is also present in other conditions such as psoriasis, keratoacanthomata, and seborrhoeic warts. We have never seen this basal keratinization in malignant states of the epidermis invading the dermis.

The areas undergoing *keratinization give the colour fluorescence of inner* root sheath keratin. This is not surprising as this keratin has also been formed without a granular layer These areas fluoresce yellow with thioflavine T and like inner root sheath keratin this is abolished by previous ribonuclease digestion.

The regions of keratinization also give a positive reaction with Baker's method for phospholipids. The blue colour is not due to nuclear protein as it remains after treatment with both RNase, and DNase.

The appearance of phospholipids in the cells becoming keratinized is probably initiated by nuclear damage resulting from the pressure on the cells. The nucleotide, cytidine triphosphate, plays an important role in the synthesis of phospholipids. If this compound is liberated during nuclear breakdown it could combine with such substances as phosphoryl choline to form a diphosphate complex which in turn reacts with a diglyceride to form lecithin and cytidine monophosphate.

Experimental pressure keratinization

The difference in chemical composition between inner root sheath and epidermal keratins was thought to be due to a different mode of keratinization. It had already been suggested by Auber that inner root sheath keratinization was initiated by pressure exerted on the turgid follicle cells. This mechanism could presumably cause keratinization of epidermal cells without the loss of nuclear protein. It was therefore thought possible that pressure on epidermal cells might cause them to keratinize, and the type of keratin produced under these conditions would resemble inner root sheath keratin rather than epidermal keratin in which nuclear protein is lost during its formation.

We investigated this hypothesis by exerting external pressure on rats tails. Continuous pressure was exerted by split rubber bungs, and the animals were killed 24, 48 and 72 hr later Skin was taken from the pressure site, and from areas above and below to make certain that there were no ischaemic effects due to interference with blood supply

These experiments showed clearly that pressure caused the *epidermal*

cells to become keratinized, and that the keratin produced resembled inner root sheath keratin. The pressure area fluoresced bright yellow with thioflavine T and the presence of phospholipids in this area was demonstrated by Baker's acid haematin method.

Pressure was then applied to the follicle cells by means of deep dermal injection of low melting point paraffin wax. The hair cycles of rats and mice were put into phase by plucking and then paraffin wax was injected into one side of the animals' backs, the other side serving as a control. A hump was raised, and by this means the pressure in the dermis increased. After 12 days the animals were killed, and skin was taken from the injected and control sides of the back. The effect of this pressure was to greatly increase the amount of inner root sheath keratin in the follicles. In fact many of the follicles had no cells remaining the whole follicle having become a tube of keratin. The keratin produced by this artificial means appeared to be the same as normal inner root sheath keratin.

SUMMARY

In normal epidermal keratinization it is thought that the keratin is produced at the granular layer At this level the nuclei of the epidermal cells disintegrate, and phospholipids can be detected by Baker's method. It is possible that these complex phospholipids are formed by the catalysing action of cytidine triphosphate, which is a nucleotide, and possibly liberated during the nuclear breakdown.

These phospholipids are thought to supply the energy for the oxidation of sulphydryl groups, and for the polymerization of the keratin precursors. The breakdown of these compounds is probably brought about by the action of phosphatases and oxidases which are present in the granules of this region. Absence of these enzymes results in abnormal keratinization such as is seen in psoriasis and in epidermal malignancy

We have shown that keratinization can be produced by pressure on epidermal cells. The keratin thus formed differs from normal epidermal keratin, but resembles very closely normal inner root sheath keratin. Also because it has been formed without a granular layer it has certain features in common with parakeratotic keratin.

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EXPLANATION OF PLATE

PLATE

Fig Normal human skin. Baker acid haematein method for phospholipids (unmodified) A band of phospholipids is clearly seen in the region f the granular layer Another band is on the surface of the keratin, and this is thought to be due t surface contamination with sebum.

Fig Normal rat tail skin. Baker acid haematein method for phospholipids (modified sebum contamination removed). The keratin in the region of the hair follicle gives negative reaction for phospholipids. The keratin away from the follicle is strongly positive.

Fig 3. Normal rat tail skin. Gomori technique for acid phosphatase The granular layer in the region of the hair follicle gives strong positive reaction for acid phosphatase, and the keratin in this area contains no phospholipids (Fig). Away from the follicle opening the granular layer becomes indistinct and fails to give positive reaction the keratin over this area gives strong reaction for phospholipids (Fig)

SOME CHEMICAL FEATURES OF EPIDERMAL DAMAGE

By KENDAL C. DIXON

We are now to consider some factors which damage epidermal cells as well as something of the properties and behaviour of these injured units of our integument.

Pathology at the present time gives lucid and revealing information on nutritional and vascular defects as well as on infective and allergic processes as causes of epidermal malady but the nature and mechanism of the underlying *cellular* disorder are frequently disregarded, probably because our knowledge of them is so imperfect.

We would like to be able to account for cellular disorder in rational terms with a similar logical regard for cause and effect as was displayed by the ghost of Hamlet's father

> The leperous distilment whose effect
> Holds such an enmity with blood of man,
> That, swift as quicksilver it courses through
> The natural gates and alleys of the body
> And, with sudden vigour it doth posset
> And curd, like eager droppings into milk,
> The thin and wholesome blood so did it mine
> And a most instant tetter bark'd about,
> Most lazar-like, with vile and loathsome crust,
> All my smooth body
>
> *Hamlet* I, v 64–73

Unfortunately our knowledge of the intimate sequence of events in epidermal injury is nothing like so precise as the ghost's!

CELLULAR DISORDER

Until cellular physiology and biochemistry are more perfectly comprehended and the fusion of these sciences with cytology and cytochemistry is achieved, a satisfactory description of even the main features of cellular disorder will not be possible.

We might consider structural and architectural damage on the one hand, and interference with chemical and physical processes on the other. However as knowledge expands, cell structure will eventually be clarified at a molecular level then the distinction between architectural and chemical

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CAUSES OF CELLULAR DISORDER

The factors which damage cells may now briefly be summarized

(1) Adverse physical environment.

(2) Lack of nutriments.

(3) Harmful substances.

(4) Living agents which may be the cells of the body itself or parasitic organisms these probably act through factors (1) (2) and (3)

(5) Inherent, inherited or mutational defects in the cell itself.

All these causes of cellular derangement injure *either* directly by disorganizing macromolecular structure, *or else* indirectly by interfering with the supply or action of micromolecules in cellular maintenance.

DISORDERS OF MACROMOLECULAR FABRIC

The fabric of injured cells shows two principal kinds of change

(i) *Katabolic change* Here derangement in the equilibrium between anabolism and katabolism causes disintegration of the macromolecular fabric. Clearly this can result from either interference with anabolism or by excessive katabolism in both cases there is a net loss of macromolecules. Dissolution of protein and of nucleic acids from injured cells are instances of katabolic change. Following thermal injury to the skin, cytoplasmic material stainable by pyronin is lost rapidly from epidermal cells (Peters, 1945) this change probably involves loss of ribonucleic acid.

(ii) *Anabolic change* Here disequilibrium results in deposition of macromolecules in excess. Fatty deposition in damaged cells illustrates this kind of disorder of intracellular equilibrium (Dixon, 1958*a*). Accumulation of viral protein in the cytoplasmic inclusion bodies, formed in epidermal cells infected by vaccinia, is another example of anabolic change.

LOCATION OF DAMAGE TO CELLULAR FABRIC

Lison (1953) in his great book *Histochimie et cytochimie animales* divides cellular constituents into three classes in respect to their visualization

(1) Substances which are insoluble in the fixative these include macromolecules like proteins and lipids.

(2) Substances which are soluble, but form part of an insoluble macromolecular edifice this class includes the nucleic acids.

(3) Soluble micromolecules which are unattached to any macromolecular complex in the fixed cell.

Methods now available only give precise information on the location of materials in classes (1) and (2). For this reason our knowledge of the

intimate nature of derangements primarily concerning micromolecules is rudimentary. On the other hand, fairly comprehensive, though still undetailed, concepts of cellular disorganization at a macromolecular level are beginning to emerge.

Even the macromolecular fabric of the cell cannot readily be observed by direct vision, since it is composed of colourless molecules with optical properties differing but little from those of their environment. The macromolecular components of the cell may, however, be visualized by attaching coloured substances to reactive groups in the cellular fabric, or by making these groups participate in the formation of coloured compounds. When the nature of these active groups is thus revealed by the reactions in which they engage, then the process of coloration gives a *cytochemical method* capable of locating individual cellular constituents with precision. Today we are going to consider a method which can be used for locating cellular protein in epidermal cells.

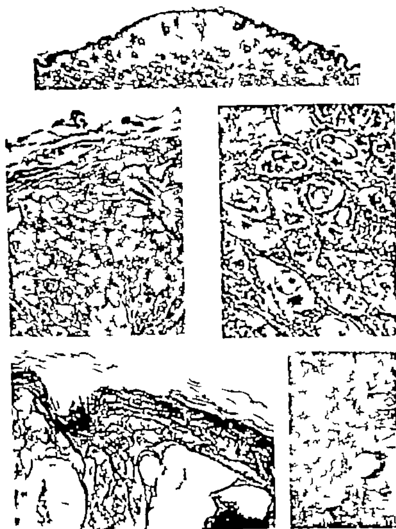
OXIDIZED TANNIN AZO METHOD FOR PROTEIN

This method utilizes the capacity of positively charged amino groups of tissues to bind the negatively charged colloidal ions of tannic acid. When sections of tissues, fixed by Carnoy's fluid, are treated with tannic acid, they turn white owing to precipitation of tannic acid on the cellular proteins (Dixon, 1958*b*). This bound tannic acid is readily demonstrated in various ways. Salazar (1921) used iron alum which forms blue-black ferric tannate by reacting with the bound tannin. Ferric chloride can also be used for this purpose (Dixon, 1958*b*). Alternatively the bound tannin may be oxidized by treatment with periodic acid to a yellowish compound, which then can be coupled with diazotized *o*-dianisidine to give a deep salmon red azo dye: this constitutes the oxidized tannin-azo (OTA) method for detection of protein (Dixon, 1959*a*). In this method the bound tannin is revealed by oxidation (probably to a quinone) followed by covalent union with a diazonium salt to form a deeply coloured dye.

Tannophilic protein in skin is readily located by the OTA method. The epidermal cells in the Malpighian layer are intensely stained. Keratinization involves loss of tannophilic properties: the stratum corneum is coloured only faintly and the cortex of the hairs is uncoloured.

After oxidation with periodic acid tanned sections of skin change to a deep salmon red on treatment with diazotized *o*-dianisidine, but without oxidation by periodic acid *only a faint pink colour is developed*. Moreover tanned sections, on treatment with periodic acid alone, are only coloured a faint yellow. The formation of the intensely coloured azo dye thus depends

PLATE I



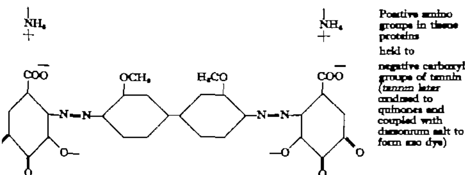
For explanation see p. 48

on the coupling of a diazonium compound with the product formed by oxidation of the tannin bound to the cellular proteins.

Pre-treatment with formaldehyde effectively inhibits the subsequent coloration of skin by the OTA method. The active groups in the tissue proteins are thus masked by formaldehyde this observation confirms the view that the loci revealed by the OTA technique are the amino groups in tissue proteins.

Untanned skin, even after treatment with periodic acid, is uncoloured by immersion in diazotized *o*-dianisidine.

The constitution of the coloured complex formed in the OTA method is uncertain it may be a compound of the following type



PROTEIN IN EPIDERMAL CELLS INFECTED BY VACCINIA

Substantial quantities of protein frequently persist in dead and dying cells (Corper 1912 Dixon, 1956). If necrosis is preceded by cloudy swelling however autolysis of protein may be rapid (Wells, 1925) in fact Dixon (1956), and Dixon & McCullagh (1957) suggested that aqueous swelling is an essential prelude to extensive autolysis of cytoplasmic protein in disordered cells. Intense aqueous swelling is a prominent feature of cells infected by vaccinia this so-called ballooning degeneration ('ballonnirende Colliquation') was first described by Unna (1894). Dixon (1959*b*) studied the content of tannophilic protein in necrotic epidermal cells in vaccinial lesions using the OTA technique the necrotic cells which remain unswollen retain their protein, but nearly all cytoplasmic protein is lost from ballooned cells which suffer aqueous swelling We will now consider the nature and mechanism of this example of epidermal disorder

Vaccinial papules were produced on the abdominal skin of young rabbits by scarifying the skin through drops of vaccine lymph.

Pl. 1 fig. 1 shows a section of a vaccinial papule stained by the OTA

method. The edge of the lesion shows the transition from normal skin through a zone of proliferation to a central region of aqueous swelling and necrosis. The cells of the Malpighian layer both *in the normal skin and at* the edge of the lesion, are well stained the central zone of the lesion contains ballooned epidermal cells which have lost most of their protein.

Pl. 1 figs. 2, 3 illustrate cells from the zone of proliferation at the margin of a vaccinial lesion stained by OTA. These cells shown at higher magnification are seen to contain cytoplasmic inclusion bodies. The inclusion bodies are even richer in protein than the rest of the cytoplasm.

In the more central parts of the lesions the epidermal cells become necrotic. Many of the necrotic cells show aqueous swelling and lose most of their protein (Pl. 1 fig 4) but some necrotic epidermal cells do not swell (Pl. 1 fig 5) and then retain abundant protein. The latter cells show coagulative necrosis as opposed to aqueous swelling In the later phases of infection the cytoplasmic inclusion bodies enlarge and multiply in some cells so as to occupy much of the cytoplasm as irregular masses rich in protein.

NATURE OF CELLULAR DISORDER IN EPIDERMAL VACCINIA

These observations on epidermal cells injured by vaccinia virus support the view that aqueous swelling is a necessary prelude to intense autolysis and loss of cytoplasmic protein. When the cells are killed without swelling, cytoplasmic protein is retained in this case rapid death of the injured cells may destroy osmotic powers so that absorption of water is prevented and extensive autolysis of protein is precluded. But if injury *is at first less* severe, so that the osmotic powers of the cells remain, then preliminary interference with synthesis may cause rise in concentration of micromolecules along with osmotic absorption of water this is followed by swelling and extensive autolysis of protein.

Among the epidermal cells in vaccinial lesions we see both *anabolic* and *katabolic* change in the macromolecular fabric. The infected cells which contain massive cytoplasmic inclusion bodies illustrate anabolic change involving deposition of abundant protein on the other hand, the necrotic swollen cells, which have lost nearly all cytoplasmic protein display intense katabolic disintegration as the final result of cellular injury

MECHANISM OF CELLULAR DISORDER IN EPIDERMAL VACCINIA

Substantial quantities of protein are incorporated into the inclusion bodies in the cytoplasm of the infected cells. The synthesis of this viral protein may utilize and deflect specific metabolites, probably amino acids, normally required for the replacement of cellular proteins which are constantly being degraded and reformed in the healthy cell.

Anabolism of some cellular proteins might thus be inhibited. The multiplication of the virus largely composed of protein may indeed be responsible for deficiency in a *particular* amino acid, so that synthesis of certain proteins may be impeded or interrupted, even though *other* amino acids are accumulating in excess. Without compensating anabolism, katabolism would soon give rise to irreparable loss of essential protoplasmic constituents if the latter are enzymic keystones responsible for synthetic processes, then macromolecular disintegration would become more general and a vicious katabolic cycle would be started. Thus viral infection may deprive the protoplasm of nutriments necessary for cellular existence, and damage may commence as a lesion of intracellular nutrition.

This tentative explanation doubtless gives but an imperfect picture of the sequence of events in epidermal injury caused by vaccinia but at least it takes account of cytochemical changes in the damaged epidermis, and it also attempts to indicate how derangement of intracellular dynamics may determine irreparable cellular disorder This supposed mode of injury moreover illustrates the principle, which almost amounts to a truism, that the causes of death and disease are limited by the mechanisms of healthy life. A similar view was admirably expressed in 1734 by Alexander Pope with the lines

> As man, perhaps, the moment of his breath,
> Receives the lurking principle of death,
> The young disease which must subdue at length,
> Grows with his growth and strengthens with his strength.

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EXPLANATION OF PLATE

PLATE

Fig Vaccinial papule on skin of young rabbit. Transition from normal epidermis through zone of proliferation to central region, where epidermal cells show intense aqueous swelling along with loss of protein. Oxidized tannin-azo (OTA) method for protein. 40.

Fig. 2. Epidermal cells from the zone of proliferation of vaccinial lesion. Cytoplasmic inclusion bodies rich in protein are visible in many of the cells. OTA. × 420.

Fig 3. Part of field shown in Fig. more highly magnified to show prominent inclusion bodies containing protein in abundance. OTA. 240.

Fig 4. Part of same field shown in Fig but more highly magnified illustrates aqueous swelling f epidermal cells with loss of protein. OTA. 420.

Fig. 5 Necrotic cells in vaccinial lesion which show no aqueous swelling; these cells retain substantial quantities of protein. The uncoloured circular area is hair cut transversely OTA. 460.

DISCUSSION

Chairman Dr W N GOLDSMITH (London)

Dr C. D Calnan (London). Can one assume that, because a cell possesses certain enzymes, they are necessarily essential to it and that it is using all these enzymes? In the skin we have come to accept the property of equipotentiality of epidermal cells and we know that cells in the skin can turn to other functions than their own, such as forming sebum, sweat or keratin.

In chloracne, the cells of the sebaceous gland normally making sebum and having the enzymes to do that, can then turn over to making keratin. Are the enzymes produced as required by cells or are they inherent in them? Another aspect that has puzzled me, is that when one sees the particularly striking demonstration by Dr Wells of the esterase in the mouth of the sebaceous gland, presumably that is there to convert some of the fat produced by the cell into fatty acid that one can identify on the surface the presence of the enzyme at that site in such concentration must have a very definite function. Since it is absent in the cell, it is evident that this chemical pathway is not used in the building up of the fat. Perhaps the build-up of the sebaceous material does not go by the same pathway as it is broken down.

Dr G C. Wells (London). It is difficult to say very much about this but I think it is clear that certain cells will produce different amounts of enzyme in different situations. In tissue culture it is possible for a cell not only to change its morphology but, in different environments, to change its predominant enzyme pattern at one time for example producing more acid phosphatase than it did initially You will remember my particular interest in the macrophage from this point of view The pilosebaceous unit is curious because of marked species differences. In the sheep the sebaceous gland shows intense esterase activity throughout its entirety whereas in the human, and particularly on the more greasy areas such as face and forehead, the mature sebaceous cells show no esterase in contrast to the sebaceous canal which shows it strongly along its lining One would like to think of this apparent concentration of esterase as having some lipolytic action, but it has never been possible to show actual lipase effect in the sense of hydrolysis of triglycerides.

Group-Captain H E. Bellringer (London). Can histochemical techniques be used as an aid to diagnosis especially in connection with a disease such as leprosy in which the diagnosis can sometimes be difficult to confirm by bacteriological and histological methods?

WELLS. I do not think it can help in diagnosis, but I would like to put the question to Dr Pearse.

DR A. G EVERSON PEARSE (London). I would not like to comment too much on that. It is a disappointing thing that diagnostic uses of histochemistry do not grow faster One of the reasons is that there are not enough people applying the techniques. It really requires much more work and I personally think it is surprising that there are as many as there are. I would like to turn back if I might to the previous question and comment generally on the matter of *enzymes shown by histochemical methods. These indicate,* of course, reactive groups present in proteins and they may or may not indicate function. Often one thinks when one makes a comparative study of various species that the whole thing is a bad joke on the part of the Almighty That is why one turns to these dehydrogenase systems very readily because there the biochemistry is so well understood. In the matter of esterases, as Dr Wells said, it is very necessary for a histochemist to try and find out exactly what the esterase is because there are a very large number of them. He has told you that he has not been able to show that it is a lipase. Many of these esterases are in fact cathepsins (or peptidases), although we are demonstrating them by making them split an ester group. One has to have a good deal of knowledge of enzyme function and this can only come from the biochemists. The faster they get on with it the better off we shall be. I would like to add—a delightful thing about these symposia is that one always learns something I am most interested in Dr Dixon s scholarly presentation and I have to admit that there are now 251 available histochemical techniques, because I had never heard of the OTA method before this morning

DR T B FITZPATRICK (Harvard) In a study of the fowl retinal pigment epithelium (Miyamoto & Fitzpatrick, 1957) we used both histochemical and cytochemical techniques to define the change in tyrosinase activity during development of the chick embryo For the histochemical technique we used ^{14}C labelled tyrosine (Fitzpatrick & Kukita, 1956) as a measure of tyrosinase activity incubating the whole intact eye. The tyrosinase activity appeared on the fourth day of development and was absent on the ninth day of development. However manometric determination of the tyrosinase activity in isolated melanin granules showed a quite different time scale the peak of tyrosinase activity occurred on the tenth day and no activity was present on the fourteenth day of development. These results indicate that it may be necessary to use both cytochemical and histochemical techniques in studying the dynamics of an enzyme change

EVERSON PEARSE. As I understand Dr Fitzpatrick, he is using and trying to compare methods which have entirely different terms of reference

Histochemically we usually investigate tyrosinase by using the old fashioned dopa oxidase method of Bloch (1917) and we might expect to find differences between that and a specific assay of tyrosinase activity as done on tissue homogenates by the biochemist. So I am not surprised at all to hear that differences were observed. With regard to the uptake of radioactive tyrosine, I do not exactly see why that should run parallel with enzyme activity though it might be proved to do so Homogenization has a very large number of limitations, as Schneider & Hogeboom (1951) who applied these techniques have very honestly admitted histochemistry is not limited in the same way It is limited because the techniques which it employs for enzymes are usually less specific and less sensitive than those of the biochemist. That is why you can see a difference. But as our techniques more nearly approach those of the biochemist we will, I hope, see much better correlation.

Dr G A. Beck (Peterborough). I should like to ask Dr Jarrett and Dr Dixon what relationship there is, if any between the pressure induced changes in the rat's tail and the changes in the cells that lose their proteins that Dr Dixon mentioned.

Dr A. Jarrett (London) The changes induced by our techniques are obviously due to external pressure on the epidermal cells, whereas those brought about by the methods of Dr Dixon are probably due to increased intracellular pressure resulting from the imbibition of water The resulting osmotic upset would interfere with the internal economy of the cell.

In our material the constituents of the cell probably remained within the cell boundaries during the process of artificial keratinization. Changes due to pressure can happen very quickly the mere pinching of a piece of living human skin can produce alterations detectable by fluorescence microscopy in less than two minutes. Therefore I think that the changes described by Dr Dixon are the result of alteration of the internal components of the cell, and in our experiments the changes are due to physical pressure on the intact living cell.

Dr Kendal C. Dixon (Cambridge). It is uncertain whether the entry of fluid into dying cells is similar to the entry of water into non living things. There are two views as to why water enters injured cells one is that when the cells die the vital process of extruding water comes to an end and the other is that, when the cells are injured, macromolecules break down and the internal osmotic pressure rises, and therefore water enters. From the work of Professor Conway of Dublin (1955) we now know that the latter view affords the more generally accepted explanation for the entry of water into dying cells.

Dr A. J E. Barlow (Huddersfield). Might I ask Dr Jarrett whether he

has examined the keratinization of the sole of the foot, because that is normally continually subjected to pressure. Does it show the same inner root sheath type of hyperkeratinization as he described?

JARRETT No palmar and plantar skin do not show inner root sheath type of keratinization. Nevertheless it does show an entirely different keratinization from that occurring on any other part of the body The cell outlines remain intact high up in the keratin layer and in this respect it differs from other keratins. Further work is required to elucidate this type of keratinization these sites are difficult to biopsy and post-mortem material is not always suitable. I have therefore only investigated these areas in the fixed state, but I can definitely say that palmar and plantar keratin is quite different from that of the inner root sheaths of the hair follicles.

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Y AND MYCOLOGY

THE SKIN FLORA AND THE STAPHYLOCOCCUS

By M. H. GLEESON WHITE

To Price (1938) we owe the simple and useful concept that the large numbers of bacteria which are to be found on human skin can be divided into a transient and a resident flora. In a long series of painstaking experiments extending over a number of years, Price was able to show that, whereas the transient flora of the skin was largely confined to the more exposed areas and could easily be got rid of by simple washing with soap and water the resident flora was only temporarily reduced by prolonged and vigorous scrubbing or the application of antiseptics.

A complete list of the transient flora of the skin would have to include not only potential pathogens such as haemolytic streptococci, staphylococci and the gram negative bacilli of faecal origin, but also the numerous species of saprophytic bacteria which abound in the environment of man. Although this transient flora is being continuously replenished by contact with the immediate surroundings, the organisms so acquired, saprophytes and pathogens alike, seem unable to establish themselves for more than a few hours on healthy human skin. Occasionally and particularly during exposure to a heavily contaminated environment, such as may occur in a hospital ward, transient pathogenic organisms may acquire temporary resident status only to lose it again when the source of contamination has been removed.

Several theories have been advanced to explain this self-disinfecting power of normal skin. For instance, Marchionini (1928, 1929*a*, *b*) maintained that the characteristic acidity of healthy human skin resulted from the acids contained in the sweat and the low iso-electric point of keratin and that this acid mantle as he called it, prevented stray bacteria from establishing themselves on the skin. This chemical theory of self disinfection was supported by other workers including Burtenshaw (1941) who demonstrated that the fatty acid fraction of ethereal extracts of human skin, hair and nails was strongly bactericidal for haemolytic streptococci, less so for staphylococci but harmless to coliform bacilli.

In contrast to this, Norton & Novy (1931 1932) advanced the view that the self-disinfecting power of the skin was simply a matter of desiccation. They showed that the rate of disappearance of cultures of bacteria applied to skin or glass was related to the speed of drying out on those surfaces.

Rebell Pillsbury Saint Phalle & Ginsburg (1950) added support to this physical theory of skin self-disinfection by showing that if drying was slowed down with the aid of an atomizer the rate of removal of bacterial cultures painted on the skin was considerably reduced. This protective power of moisture was even more strikingly demonstrated by applying cultures of coliform bacilli to the skin of volunteers who then remained in a hot room of high humidity for some hours after The coliform bacilli established themselves on the skin of these volunteers and could be recovered from there with ease from the majority of them for as long as three weeks afterwards.

These conflicting theories of skin self-disinfection have now been partly reconciled by the work of Ricketts, Squire, Topley & Lilly (1951) who showed that, depending upon the species of bacteria used, either desiccation, chemical action or both might play a part in their removal from the skin. By covering the test areas with either a nylon dressing which was freely permeable to water vapour or with polyethylene sheeting which effectively prevented any evaporation from the underlying skin, they were able to study the effect of moisture on survival with much greater accuracy than had been achieved before. Under the nylon dressing haemolytic streptococci, coliform bacilli and pseudomonas disappeared in less than a day with staphylococci surviving for as long as three days. Under the polyethylene sheeting the haemolytic streptococci again only survived for less than a day the other organisms lasting a day or more with the coliform bacilli and pseudomonas actually multiplying on the moist skin surface. From these experiments they concluded that although desiccation was an important factor in the removal of gram-negative bacilli from the skin, and to a lesser extent staphylococci, it was not responsible for the destruction of haemolytic streptococci since they died out equally quickly on moist or dry skin. Following this, they fractionated sebum and demonstrated that haemolytic streptococci were highly sensitive to the unsaturated fatty acid fraction, and in particular to oleic acid, whereas staphylococci were less affected by it, and gram negative bacilli hardly at all.

Blank & Coolidge (1950) have drawn attention to another possible factor in the self-disinfecting mechanism of the skin During a study of surgical scrubbing-up procedures, they re-examined Miller's (1940) hypothesis that the reduction of the skin flora that appears to follow the application of quaternary ammonium compounds was not due to any bactericidal action but to the mechanical sealing of the bacteria to the skin by a thin residual film of these compounds which could be easily destroyed with soap or alkali with the release of the still viable bacteria. Blank & Coolidge however demonstrated that the quaternary ammonium compounds acted

by lowering the pH of the skin below the iso-electric point of keratin which built up a positive charge at the skin surface, thus attracting instead of repelling the negatively charged bacteria. The subsequent application of soap or alkali immediately restored the normal negative charge at the skin surface and allowed mechanical removal of the bacteria to proceed in the usual way *From these observations they concluded that the normal* negative charge at the skin surface probably played a part in ridding it of transient organisms.

From this brief summary of the more important studies of the self disinfecting power of normal skin it is clear that the mechanisms involved vary greatly from one species of bacteria to another While haemolytic streptococci would appear to be rapidly destroyed by the unsaturated fatty acids contained in sebum, desiccation would seem to be of prime importance in the ridding of the skin of gram-negative bacteria the removal of staphylococci, however is probably helped by both desiccation and chemical action. Nevertheless there is a flaw in all these arguments they are all based on the results of experiments in which very large numbers of bacteria were applied to skin already carrying its normal quota of resident organisms. More subtle methods of investigation will have to be used before we can arrive at a complete understanding of the working of this vital defence mechanism against infection from without.

In contrast to the transient flora of the skin which, by its highly variable composition, reflects its origin in man's constantly changing bacterial environment, the *resident flora is found to consist of very large numbers of* some half-a-dozen species of bacteria. Though the number and distribution of these different organisms is affected to some extent by such variables as the climate and the age, hygiene, clothing and general activities of the individual, and differs greatly from one anatomical site to another the resident flora as a whole can be regarded as a mixed population of a few species of saprophytic bacteria in equilibrium with their immediate environment.

The heaviest yield of aerobes is obtained from the intertriginous zones and the sebaceous skin of the face scalp and upper chest, and the poorest growth from the non hairy skin of the trunk. If the anaerobic flora is also taken into account, a direct relationship can be shown between the distribu tion of the resident flora and the sebaceous glands.

Surprisingly enough, lack of bathing does not raise the total flora to any appreciable extent, though there may be a temporary increase in the number of organisms in the intertriginous zones. Temporary increases may also occur following heavy sweating brought about by exercising under conditions of high humidity Conversely the total skin flora is said by

Rebell & Pillsbury (1954) to be depressed during the winter months, a fact which they attribute not only to a lowering of the skin temperature, but also to a possible reduction in the degree of hydration of the horny layer

The resident flora consists for the most part of aerobic gram-positive cocci and lipophilic diphtheroid bacilli, with *Staphylococcus albus* the predominant organism and other species such as *M. candidus* and *M. flavus* present on the skin of some individuals and not of others. The principle anaerobic member of the resident flora is the acne bacillus which, according to Evans, Smith Johnston & Giblett (1950), is present in far larger numbers than has hitherto been supposed.

Estimates of the number of organisms present on various areas of normal human skin are as diverse as the methods which have been used to obtain them. Although there are probably considerable differences in the density of the bacterial population on adjacent areas of skin and between the same areas in different individuals, the results obtained from the repeated sampling of the same area in the same person have sometimes been remarkably constant over long periods of time. Price (1938), for instance, estimated that the total aerobic flora of the hands and forearms of sixteen subjects ranged from 2,500,000 to 16,000 000, each individual maintaining a characteristic count over many months, his own remaining constant at 9,000,000 for as long as nine years. Other workers have not achieved such consistent results as these. Evans, Smith Johnston & Giblett (1950), for example, obtained bacterial counts ranging from as low as 70 to as high as 200,000 organisms per square centimetre from serial skin scrapings from the back of the same subject. How much of this wide variation was due to the difficulty of standardizing the method of sampling used and how much to actual changes in the density of the resident flora is difficult to assess. Again the average number of aerobic bacteria on the hands and forearms has been variously estimated at 170 253 600 and 3200 per square centimetre by four separate groups of workers (see Pillsbury & Kligman, 1954).

Of greater interest, perhaps, than the numbers present, is the precise location of these organisms which are so difficult to remove from the skin even by vigorous scrubbing or the application of antiseptics.

It is a commonplace that bacteria can rarely be recognized with certainty in ordinary stained sections of skin. Lovell (1945) overcame this difficulty by incubating his samples of skin for several hours before fixing them and cutting sections in the usual way This ingenious though simple technique allowed the bacteria present to multiply *in situ* before the fixation of the tissues, thus rendering their subsequent location in the sections a comparatively easy matter Using this technique in a variety of ways on a large number of skin samples, Lovell was able to establish that although there

might be an occasional organism in the *stratum corneum*, the bulk of the resident flora was to be found deep in the crypts and hair follicles and in the sebaceous glands and their ducts On no occasion did he find any bacteria in the sweat glands. Therefore, it is not surprising that with the resident flora so situated it is virtually impossible to render the skin sterile by scrubbing or the application of antiseptics. Furthermore, Price (1938) has shown that even after the most prolonged and vigorous scrubbing of the hands, the resident flora returns to its normal level in a matter of days, this recovery period being shortened to a few hours if sweating is induced by the wearing of rubber gloves.

So long as the skin remains healthy and intact its surface will be fully occupied by the resident flora, leaving little opportunity for colonization by transient organisms. However when any breach in the continuity of the skin exceeds the limit of immediate repair or the self-disinfecting mechanism of the skin is deranged by some eruptive process, colonization by potentially pathogenic members of the transient flora is usually rapid and it is not long before the saprophytic resident flora is driven out of the area.

Nevertheless, the isolation of pathogenic members of the transient flora from a skin lesion does not necessarily imply that these organisms are playing an active part in the production of the lesion. For instance, a burn or an eczematous eruption may be teeming with staphylococci which, as far as can be shown in the laboratory are identical with a strain of staphylococcus just isolated from a fatal septicaemia but without there being any evidence that their presence is in any way delaying the healing of the burn or prolonging the eczematous condition of the skin. Conversely strains of staphylococci which would otherwise be classed as non-pathogenic are not infrequently isolated under circumstances which make it impossible not to regard them as the prime cause of the lesion. This is only one example in the many gaps in our understanding of the behaviour of staphylococci seventy five years after Ogston (1881) carried out his pioneer research into the causes of suppuration.

Two years after Ogston first called these organisms staphylococci in his classic papers on Micrococcus poisoning (1881 1882) Rosenbach, using Koch s recently introduced gelatine culture medium, succeeded in obtaining pure cultures of staphylococci. These he divided into two species, *Staphylococcus aureus* and *S albus* on the basis of pigment production, and to these a third, *S citreus* was added the following year by Passet (1885). In the same year Garré (1885) and other workers placed beyond doubt the pathogenicity of staphylococci by producing pyogenic lesions in themselves and others by the inoculation or the rubbing of staphylococcal cultures into the skin.

In 1894, van der Velde reported on the toxicity of staphylococcal filtrates and during the next fifteen years most of the toxic properties of such filtrates recognized today were observed and described by other workers. After that, general interest in staphylococci and their toxic properties seems to have flagged until 1928 when the Bundaberg disaster in which twelve children died after being inoculated with a diphtheria toxin-antitoxin mixture which had become contaminated with staphylococci, led Burnet to reinvestigate the whole problem anew. During the next three years Burnet (1929, 1930, 1931) published a series of papers in which he described the production and investigation of a potent exotoxin in staphylococcal culture filtrates whose *in vivo* and *in vitro* activities could be fully neutralized by antisera prepared against it in animals. He also demonstrated that this toxin was elaborated *in vivo* in animals infected with staphylococci.

In the years immediately following Burnet's stimulating lead many more papers, some of them contradictory particularly in regard to the haemolytic activities of these culture filtrates, were published by other workers. Much of this confusion was cleared up by Glenny & Stevens (1935) when they identified two antigenically distinct toxic components with different *in vivo* and *in vitro* activities, either or both of which might be present according to the strain of staphylococcus used to prepare the culture filtrate. Two further toxic components, distinguishable by their haemolytic properties, have since been described (Smith & Price, 1938; Williams & Harper, 1947).

About this time the coagulation of plasma by staphylococci, originally described by Loeb (1903–4) some thirty years before, began to receive attention as a possible factor in the establishment of both local and pyaemic staphylococcal infections. In 1937 Cruickshank introduced the coagulation of plasma as a routine test for the differentiation of pathogenic from non-pathogenic strains of staphylococci. Since then this test has been widely and successfully used and the term 'coagulase-positive' has now been accepted by most bacteriologists as synonymous with pathogenicity.

The outbreak of the Second World War brought problems of wound infection to the fore again. The lack of any practical method of distinguishing one strain of staphylococcus from another made the study of staphylococcal cross infection of wounds impossible, but this obstacle to the epidemiological study of the staphylococcus has now been partly overcome by the use of 'phage typing.

With the release of penicillin for general use in 1946, it seemed as if the end of serious staphylococcal disease might be in sight. However, the rapid emergence of resistant strains of staphylococci has created fresh problems, and new forms of acute staphylococcal infection have appeared which are

proving as difficult to treat as the fulminating septicaemias of the pre-antibiotic era.

Staphylococci are among the easiest of organisms to cultivate, and, apart from an occasional CO_2-sensitive, dwarf colony variant which may not be easily recognizable on plate cultures incubated in air alone, their isolation and identification from acute staphylococcal lesions usually presents no difficulty (Hale, 1951 Goudie & Goudie, 1955 Thomas, 1955). The introduction of salt-enriched medium has increased the isolation rate from faeces and contaminated foodstuffs and the use of media containing phenolphthalein phosphate has made the detection of nasal carriers and environmental contamination with pathogenic staphylococci very much easier (Barber & Kuper 1951).

By far the greatest single source of pathogenic staphylococci is the human nose. Over half the population are nasal carriers of these organisms and the skin of the face and hands of these carriers is being continuously contaminated with their own nasal secretions which often contain very large numbers of these organisms (Miles, Williams & Clayton-Cooper 1944). This in turn inevitably leads to wholesale contamination of their clothing and bedding at the slightest movement of which, showers of infected dust-particles rise into the air constituting a much more dangerous and effective vehicle for the spread of these pathogenic organisms than droplet nuclei from the expired air of these carriers (Duguid & Wallace, 1951 Hare & Thomas, 1956 Hare & Ridley 1958). The faeces of a nasal carrier is another important source of staphylococcal contamination which is often overlooked. The contribution of infected burns and skin eruptions to the wholesale contamination of our hospitals needs no comment.

The toxic properties of staphylococcal culture filtrates vary considerably according to the strain of staphylococcus used and the method of culture employed. Of the four haemolysins which may be present in such culture filtrates, the α lysin is generally regarded as the most important as far as human infections are concerned. This toxin is not only haemolytic, but also dermo-necrotic and lethal to rabbits and mice it is neutralized by specific antisera prepared by the active immunization of rabbits with the toxin or its toxoid.

The production of the β-lysin is more characteristic of strains of staphylococci isolated from animals. This toxin exhibits the hot-cold type of haemolysis, is not dermo-necrotic, and only lethal to rabbits if given in large amounts. It is antigenically distinct from the α lysin and can also be neutralized by its own specific antisera.

The γ- and δ-lysins are both dermo-necrotic but differ antigenically and in their haemolytic behaviour little else is known about them at present.

The majority of pathogenic staphylococci produce at least two leucocidins, one of which is almost certainly the α lysin and is only active against rabbit and not human leucocytes. The other known leucocidin was first described by Panton & Valentine (1932 see also Valentine, 1936), is antigenically distinct from the α lysin, and destroys both human and rabbit leucocytes. It has since been shown that agglutination of the leucocytes precedes their destruction and this has been used as an indirect method of estimating the leucocidin content of culture filtrates (Weld & Mitchell, 1942).

There are also other substances present in culture filtrates of pathogenic staphylococci which, although not toxins in the strict sense of the word, probably play a part in the establishment of staphylococcal infections. The most important of these is the well-known clotting factor staphylocoagulase. Originally thought to be a thrombin like substance acting directly upon plasma fibrinogen, it is now regarded as a kinase which activates a prothrombin-like factor present in the plasma itself which then reacts with fibrinogen to produce the fibrin clot. The presence or absence of this prothrombin like factor determines whether the plasma of any given species of animal can be coagulated by staphylocoagulase. Human, rabbit and horse plasma is readily clotted by almost all strains of staphylococci pathogenic to man, whereas guinea-pig, mouse, rat or fowl plasma is resistant. The fact that there are a few strains of coagulase-positive staphylococci which can coagulate guinea pig plasma and an occasional strain that will clot mouse plasma would seem to imply that some staphylococci may produce more than one type of staphylocoagulase and that the prothrombin-like factor in plasma is probably species specific (Smith & Hale, 1944 Duthie & Lorenz, 1952) The generally accepted view that the pathogenicity of staphylococci is closely linked with staphylocoagulase production receives support from the fact that guinea pigs are not resistant to experimental infection with those few strains of staphylococci which are able to coagulate guinea-pig plasma. Furthermore, the virulence of a coagulase-positive strain of staphylococcus for a normally resistant species of animal can be raised by suspending the infecting dose of staphylococci in coagulable plasma obtained from a susceptible species. The study of antibodies to staphylocoagulase found in man and experimental animals is not far advanced enough to permit an assessment of their significance in staphylococcal disease.

Culture filtrates from pathogenic staphylococci may also contain varying amounts of enterotoxin, fibrinolysin hyaluronidase, lipase and protease. With the exception of the direct relationship between the enterotoxin and staphylococcal food poisoning the part played by these substances in the production of staphylococcal disease is at present uncertain.

The original observation by Russ (1916 see Wright, 1942) that the intravenous injection of a lethal dose of staphylococcal culture filtrate in the anaesthetized cat or rabbit caused an initial fall of blood-pressure with a temporary return to a point above the normal followed by a terminal fall, was confirmed and extended by Burnet and others, who also demonstrated the neutralizing power of staphylococcal antitoxin (Burnet, 1930 Kellaway Burnet & Williams, 1930). At *post mortem* they too found ample evidence that these animals died of acute right-sided heart failure arising from pulmonary hypertension. Furthermore, they were able to produce the same effects by the intravenous injection of living staphylococci and demonstrated free staphylococcal toxin in the pleural and pericardial effusions found at *post mortem* in these animals. The resemblance between the toxic action of these staphylococcal filtrates was subsequently investigated by other workers who demonstrated the release of histamine in the lungs. This, however would be regarded today as only evidence of cell damage and not as a successful demonstration of the substance responsible for the production of pulmonary hypertension this still remains to be discovered. The recent report by Ogasawara & Tanaka (1958) that mice given small doses of staphylococcal toxin intranasally die with extensive haemorrhagic consolidation of the lungs and that this can be prevented, not only with antitoxin, but also with the hypotensive drugs tetraethylammonium bromide and chlorpromazine, is an important step forward in the search for this pressor substance.

Staphylococcal toxin also has a pronounced nephrotoxic activity damaging the glomerular capillaries and the proximal convoluted tubules with production of albuminuria, oliguria and uraemia.

The dermo-necrotic property of staphylococcal toxin, although used extensively for toxin and antitoxin titrations, has been little studied of recent years.

Most strains of staphylococci pathogenic to man kill rabbits and mice, but the time and mode of death vary greatly with the size of the inoculum and the route by which it is given. For instance, the subcutaneous injection of 200 000,000 staphylococci into the flank of a rabbit only produces a localized abscess from which the animal recovers completely whereas the intravenous injection of a tenth of that number of staphylococci will kill a rabbit in under 24 hr., the animal dying of an acute toxaemia with haemorrhagic exudates into its serous cavities. Another tenfold reduction of the dose, again given intravenously produces little or no immediate toxaemia but the rabbit will die of cachexia in one to six weeks with multiple abscesses in the kidneys and other organs. Similarly the route of inoculation in mice largely decides the pattern of staphylococcal disease produced. For

example, the intraperitoneal injection of 200 000 000 staphylococci will kill mice in 7 hr., the animals dying of an acute haemorrhagic pulmonary oedema. Half that dose by the same route has little immediate effect and such mice as eventually succumb die after a week or ten days of the same pyaemic lesions as mice given a tenth of that dose of staphylococci intravenously Moreover animals actively immunized with staphylococcal toxin may escape sudden death from toxaemia when challenged with large doses of living staphylococci only to die a week or so later from typical staphylococcal pyaemia.

Smith, Wilson, Hummer & Godfrey (1958) have recently reinvestigated the effect the route of administration has upon the course of experimental staphylococcal infection in mice and have come to the interesting conclusion that *no matter which route is employed the same critical lethal titre in the region of 1 000,000 000 viable cocci per mouse, is always reached before death occurs.*

From the results of these and many other similar studies two extreme types of experimental staphylococcal disease in animals emerge each with its counterpart in man. One is an acute toxaemia running a rapid course with death from right-sided heart failure caused by a mounting pulmonary hypertension the other is a slow disorganization of the animal's vital processes arising from the progressive destruction of essential tissue by the formation of multiple staphylococcal abscesses.

In man, the swiftly fatal haemorrhagic staphylococcal bronchopneumonia, at one time rarely seen except as a lethal complication of virus influenza, but now becoming an increasingly common manifestation of staphylococcal cross-infection in hospital patients, and the fulminating staphylococcal enterocolitis which may follow the injudicious use of the broad spectrum antibiotics, are examples of the acute toxaemic type of staphylococcal disease. The progressive type of pyaemic disease, such as the minute and sometimes hypothetical skin lesion giving rise to an acute osteomyelitis followed by a succession of metastatic abscesses in other parts of the body is happily much less common now than in the pre-antibiotic era, though it may well return again if antibiotic resistant staphylococci continue to spread through the population at large.

Between these two extremes lies the main mass of staphylococcal morbidity—the styes, boils and carbuncles the recurrent attacks of furuncules and sycosis barbae and the outbreaks of impetigo—about the pathogenesis of which we seem to know so little

Evidence has rarely been forthcoming that strain variation in virulence and metabolic requirements are responsible for the wide differences in the types of lesion produced by this single species of organism or of the chronicity

of some of these infections or their distribution in the community. A notable exception is the report by Parker (1956) of a very high incidence of phage type 71 staphylococci (a Group II strain with some unusual cultural characteristics) which he found during the investigation of an extensive and prolonged epidemic of impetigo in schoolchildren. There is also some evidence that phage type 80 includes some unusually invasive strains of staphylococci.

The results of numerous studies of the antibody response in man and animals to natural or experimental infection with staphylococci, have, on the whole, been as disappointing as vaccine, toxoid or serotherapy in the treatment of human staphylococcal disease. Nevertheless, the recent report by Johanovsky (1958) that toxoid immunization during pregnancy reduced the incidence of staphylococcal infection in both mother and infant alike, and that this appeared to be independent of the α-antitoxin content but directly related to the anti-leucocidin titre of maternal and cord blood, should revive interest in the role of leucocidins in staphylococcal disease.* In this connection the thirty year-old, but hitherto unconfirmed, report by Lyons (1927) that staphylococci in very young cultures are encapsulated and that the anti-capsular antibodies that can be produced in rabbits by immunizing with young cultures behave like anti-leucocidins, might well repay reinvestigation on the lines so successfully employed by Gladstone (1946), Cromartie, Bloom & Watson (1947) and Smith, Keppie, Ross & Stanley (1954) in the elucidation of the pathogenesis of cutaneous anthrax. Nor should Bøe's (1945) attractive hypothesis that it is the degree of hyper sensitivity of the host's tissues that determines the character and course of a staphylococcal infection be overlooked.

To sum up, it can be fairly said that although we now know a great deal about the cultural characteristics and toxic products of staphylococci, their source and distribution in man and animals and their modes of spread, our experimental techniques are still too crude to elucidate the factors which determine the onset and course of staphylococcal infection in the individual.

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CANDIDA IN KERATIN OF NAIL FOLDS

BY C. HOWARD WHITTLE AND G AUSTIN GRESHAM

Candida grows in the keratin of the horny layer where it may remain as a saprophyte or may assume invasive characters and set up inflammation. Little is known of the conditions which favour the change from the saprophytic state, except that the organism has been found associated with lesions most frequently in parts of the skin which are unusually moist, such as the intertriginous surfaces in the groins and toes and under the breasts in women.

Candida albicans is very rarely found on normal skin (Benham & Hopkins, 1933 Mackinnon, 1946 Croft & Black, 1938) and cannot be regarded as a regular inhabitant, though it was found by Benham & Hopkins in the alimentary tract in 18 per cent of normal persons and even more frequently by other workers. Of the various species of candida recovered from the skin, *C albicans* is by far the commonest in actual lesions, and on this and animal inoculation tests—Redaelli (1924) Winner (1958*a*) Gresham & Burns (1959)—it stands out sharply from the others. But other strains or species are not necessarily completely devoid of invasive power and apparently may on occasions be responsible for producing lesions, for example, Raubitschek (1946) Floch & Mailloux (1957) Benham (1957) Whittle, Moffatt & Davis (1959).

We have confined our attention to candida infections of the nail folds. This is what we think happens when infection takes place.

1 SOFTENING OF THE KERATIN BY MACERATION

This seems to be essential if the candida is to gain a footing. The very high incidence in women in this country (96/104, Whittle *et al.* 1959, see Table 1 Hellier 1955 Frain Bell, 1957) suggests that primary paronychia is an occupational disease of housewives because of the repeated wetting and degreasing to which they subject their fingers. In Tel Aviv Ferchenfeld (1958) found 90 per cent of his patients were housewives. German and French workers note a similar preponderance in housewives. In the United States, however where many wet household duties are done by labour-saving machines, the disorder is less often seen in women though it occurs in men in occupations which involve repeated wetting of the fingers (Kingery & Thienes, 1925 Thienes, 1929 Sutherland-Campbell, 1929 Scherr 1958).

PLATE 1

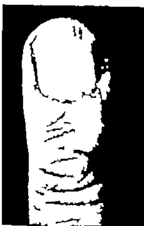
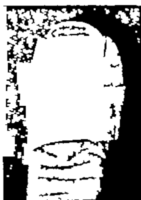


Fig.



Fig

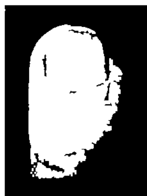


Fig 3



Fig 4



Fig 5



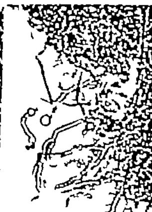
Fig. 6

For explanation see p 73

PLATE 2



Fig



Fig

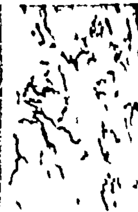


Fig. 3

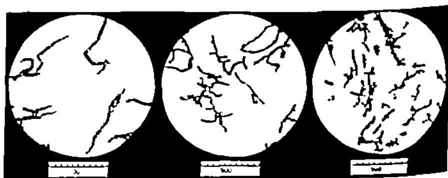


Fig. 4

Fig. 5

Fig. 6

For explanation see p. 73

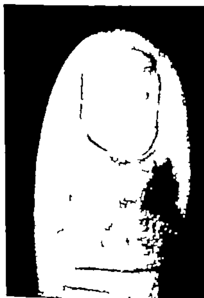


Fig.



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Fig 3

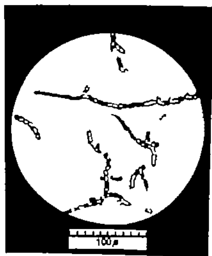


Fig. 4

For explanation see p. 73

Table 1 *Likely contributing factors in primary paronychia (104 cases)*

Indirect	Sex	Female 97 cases	
		Male 7 cases	
	Age	(57 cases) in fifth and sixth decade	
	Circulatory disturbances	Hyperidrosis	28 cases
		2. Chilblains	
		3 Dead fingers	
		4. Arthritis of fingers	
		5 Constriction from wedding ring	
Direct (trauma)*		Wet and/or rough work	89+ cases
		2. ?Thimble (starting on this finger)	7 cases
		3 Blow crush, splinter puncture wound, hang nail	5 cases
		4. Drastic manicure to nail folds	cases
		5 Biting, picking scratching, rubbing	6 cases

There is some overlapping in these figures when more than one factor was recorded.

The ease and speed with which candida can gain a footing and grow in the macerated nail fold has been shown in a simple experiment by one of us (C.H.W *loc. cit.*), in which a healthy fold was macerated by occlusion with waterproof adhesive tape (Pl. 1 figs. 1 2, 3). Though no organisms were knowingly introduced, spontaneous colonization of the keratin by candida (in this case *C guilliermondi*) was demonstrable in a week or so The fold remained sterile for two weeks but was heavily infected after three weeks (fifty colonies of candida on slope culture, Pl. 1 fig 4) Similar results were obtained in another nail fold in the same subject. As a source of infection the plaster itself is unlikely since nine different samples failed to grow candida.

2. MECHANICAL INJURY

This may be single, sudden and violent, as a blow or crush or splinter or more often mild unnoticed and repeated as in housework, gardening, etc. (see Table 1).

3 CIRCULATORY DISTURBANCES

There is evidence of disturbed circulation in the fingers in a small proportion of cases (28/104, see Table 1) an extension of the series may reveal a higher incidence.

4 INFECTION

The onset of symptoms is usually sudden and the inflammation acute (see Pl. 3 fig. 1). The organisms found are those normally present in the bowel and/or mouth, and the infection is probably introduced by con tamination of the fingers from the alimentary tract (Marten, 1959). The danger of wide-scale faecal contamination of the fingers is well known, and public health authorities are attempting to reduce it by displaying notices

in public lavatories and by providing better washing facilities therein. In our series the right middle finger showed a significantly high incidence as the first digit to be affected, which supports the suggestion of fæcal contamination, since in cleansing the perianal skin after defæcation it is the one finger most likely to be contaminated (Whittle *et al.* 1959).

The candida no doubt reaches the fold in spore or yeast form. But mycelium may soon develop and mycelium is frequently present in established infections (Pl. 3 fig 2). In some cases only mycelium is found and in one case, *loc. cit.*, a closed abscess of the nail fold (Pl. 3 fig 1) showed only mycelium in the pus and only pure *C albicans* was grown on this and several subsequent occasions, both from this and from other affected folds.

The mycelial form of candida is also found in scrapings from intertriginous lesions such as tinea cruris and intertrigo of the toe clefts, and it is easily mistaken for one of the dermatophytes, such as *Epidermophyton floccosum* and *Trichophyton rubrum* (Pl. 3 figs. 2, 3 4) until the culture reveals the true nature of the infection.

Kapica & Blank (1957) have shown that *Candida albicans* will grow *in vitro* on keratin with glucose and develop mycelium, utilising the keratin as its sole source of nitrogen. It took about 30 days to produce mycelium in any quantity In our experimental nail fold infection with *C guilliermondi* mentioned above, only yeast forms were found in the scrapings in the first 30 days, up to the time the report was made but abundant mycelium appeared after 34 days from the start, and persisted. *C guilliermondi* and *C parapsilosis* have since been recovered at frequent intervals and are still present, though other fungi, possibly contaminants, have appeared. The mycelium has been present in abundance in the nail fold keratin for the 12 weeks since first observed (*Pl.* 1 figs. 5 6 *Pl.* 2, *figs.* 1–6). Histological sections show that the mycelium is *invading* the keratin (Pl 2 fig 3) and not merely growing on the surface. *C guilliermondi* can apparently produce perionychial lesions (Raubitschek, 1947 Floch & Mailloux, 1957 Whittle *et al.* 1959 Benham, 1957) However before drawing any final conclusion we hope to repeat this experiment.

The nail fold will contain very little glucose and will be subject to changes of pH and oxygen tension as well as to alterations in the keratin, the composition of which is influenced by numbers of factors both internal and external (Barlow & Chattaway 1955 Barlow 1958 Jarrett, 1958). It seems likely that some of these changes may greatly favour mycelial development. Lowered temperatures (Rees, 1870 Hansen, 1886) and lowered oxygen tension (Wickerham & Rettger 1939), certainly increase this tendency

We think that the mycelium may play a part in candida s power to invade

keratin and produce lesions, and in this respect candida behaves like the dermatophytes. We know that this idea is in conflict with some accepted views on the subject, which rest by analogy on the behaviour *in vivo* of *Cryptococcus* and *Histoplasma* (for example, Winner 1958*b*) but these organisms differ from candida in that they evince no affinity for keratin.

The generally accepted belief that the *Monilia* genus is primarily unicellular in nature is shown to be untenable by the demonstration that this fungus may produce true hyphae which arise by septation of the filament rather than by a succession of polar buds and that a division of labour exists among the cells of this plant, as is shown by the utilization by developing chlamydospores of food produced in and transported from the hyphal cells (Wickerham & Rettger 1939) thus candida is closely linked with the moulds.

Biochemical studies support this view Foster (1947) writing on moulds contends that the fermentation of sugars by the organism is a sign of disordered metabolism. In the artificial conditions of the laboratory sugars are provided in amounts far in excess of natural requirements the incomplete utilization of these sugars results in the accumulation in the media of incompletely oxidized products such as the organic acids, whereas the natural end products of a healthy metabolism, as in other plants, should be carbon dioxide and water We therefore regard the yeast phase, or unicellular form of budding, as the more primitive, signifying a degenerate streak in an otherwise well-organized multicellular plant.

We have described elsewhere the production artificially of perionychial inflammation, of a mild and transient character following simple maceration and occlusion of a previously healthy nail fold (Whittle & Gresham, 1959). The conditions which permit the organisms hitherto saprophytic to assume invasive powers are discussed and, as Duncan (1947) suggested, may well be bound up with a temporarily diminished resistance of the host, though what these changes are and how they affect the seat of infection is not yet apparent. Local sensitization of the tissues, as Henrici (1940) thought, may be one factor necessary for the production of disease.

SUMMARY

Clinical and experimental evidence has been adduced to show that candida can invade nail fold keratin *in vivo* and that it does so in the mycelial phase, and is in this respect more like a dermatophyte than a yeast.

ACKNOWLEDGEMENTS

We wish to thank Dr Jacqueline Walker London School of Hygiene and Tropical Medicine, for her help in identifying the Candidae, Dr M H

Gleeson White of the Cambridge University Department of Pathology for the bacteriological examinations, and the authors, the editors and publishers of the *British Journal of Dermatology* for permission to reproduce Table 1 and Pl. 3 figs. 1 2

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EXPLANATION OF PLATES

PLATE *Experimental nail-fold infection with Candida*

Fig. Nail fold of experimental subject before maceration.

Fig. 2. The same occluded with water-proof adhesive tape

Fig. 3. The same after days maceration.

Fig. 4 Slope culture from nail fold taken after days maceration heavy growth of *Candida guilliermondi*.

Fig. 5. Mycelium in KOH mount of keratin from experimental nail-fold infection at 34 days. (Photomacrograph 20.)

Fig. 6 Mycelium as in Fig. 5 but high power. 700

PLATE *Experimental nail-fold infection with Candida (continued)*

Fig. 1. Mycelium in later sample from same fold. 500

Fig. Mycelium in later sample from same fold showing chlamydospore-like structures. 500

Fig. 3. Histological section showing mycelium invading the keratin. 900.

Fig. 4. Camera lucida drawing of details of the mycelium in keratin an early stage.

Fig. 5. Camera lucida drawing 4 weeks later

Fig 6. Camera lucida drawing but in late stage of another experimental fold similarly affected

PLATE 3. *Paronychia (perionychia) in patients*

Fig. Paronychia in early acute stage, with closed abscess and intact cuticle only mycelium present, with pure culture of *Candida albicans*.

Fig. 2. Mycelium in KOH mount of nail fold scrapings from paronychia—*C albicans*. Photomicrograph 5 7

Fig. 3. Details of mycelium from paronychia—*C albicans*. Camera lucida drawing.

Fig 4. Mycelium from intertrigo of toes—*Epidermophyton floccosum* note resemblance to mycelium of *Candida*.

TISSUE INVASION BY CANDIDA

By G AUSTIN GRESHAM AND M. BURNS

INTRODUCTION

An investigation of pathogenicity within the genus candida, is complicated by some disagreement over the most reliable criteria for species differentiation. The situation has changed but little over the last two decades. There are three things which seriously hamper the classification of the yeast like monilias. The first is the dearth of general knowledge of the biology of these organisms the second is the lack of a conception of the limits within which a single species may vary and the third is the absence of the concise but comprehensive definition of the genus (Wickerham & Rettger 1939).

Table 1 *Some possible biological relationships in fungi*

Saprophyte	Symbiont	Parasite
Living on dead organic matter	Close association of two dissimilar organisms to the advantage of both	Living on or in an organism at its expense. Parasites may have the properties of 1 Invasion 2. Survival in tissue 3 Multiplication in tissue 4. Toxin production

Various species of candida are occasionally found on human skin and often on mucosal surfaces in both situations they are probably saprophytic. Our concern is with those factors which have been studied in experimental animals and which enable candida to penetrate into tissues and produce deep-seated disease.

METHOD

Much that has been written about various facets of candidal pathology is based upon experiments using one species of candida and one animal species. It is only when many candidal species are used in a variety of animals and when histopathological, mycological and immunological studies are made together that one obtains a comprehensive picture of the problems involved in the production of disease by members of the genus candida (Ashford, 1916 Redaelli, 1924).

Accordingly species of candida were obtained from human sources they were *Candida albicans tropicalis parapsilosis* and *guilliermondi* saline suspensions were injected into rabbit, rat, mouse and guinea pig It was essential, at the outset, to be certain of the taxonomic features of the species used The organisms were classified according to macroscopic and

macroscopic growth characteristics on a variety of media, by sugar fermentation and by animal pathogenicity tests details are set out in the Appendix.

The size of the dose used to inoculate animals was computed turbimetrically the number of organisms in a given suspension was remarkably constant when frequently checked in a haemocytometer Saline suspensions were prepared using 24 or 48 hr cultures. Aged cultures of organisms used after repeated subculture frequently lost their pathogenicity in the usual dosage.

CANDIDA ALBICANS INFECTION IN THE RABBIT

The intravenous inoculation of about 100 million *Candida albicans* into young or adult rabbits killed them in about 3 days. The animals became listless, dyspnoeic and died occasionally epileptiform fits occurred. Necropsy revealed swollen kidneys studded with minute white dots (Pl. 1 fig. 1) and abundant mycelium was found in smears of bladder urine. The blood urea was frequently as high as 200–300 mg per 100 ml. and histological examination of the kidneys showed capillaries packed with blastospores and mycelium and surrounded by an infiltrate of polymorphonuclear leucocytes into which mycelial strands extended in places (Pl. 1 fig 2). Rabbits killed on the second day of infection show less inflammatory reaction and less mycelium formation, than those which received a smaller dose of *Candida albicans* and survived several days showing streaks of a chronic purulent inflammatory infiltration extending from cortex to medulla.

Candida albicans spreads from its primary intravascular location into Bowman s capsule and thence down the nephron by first penetrating vessel walls. This penetration is achieved by mycelial strands which are often extensively formed (Pl. 1 fig 3). Histochemical studies of this mycelium have shown that it is rich in protein and ribonucleic acid this favours the view that it is the rapidly growing colonizing phase of the organism. Anoxia may be one of the stimuli which enhances the profuse mycelial outgrowth since many small capillaries in the areas of inflammation contain fibrin thrombi. Whatever the stimulus be, and we are still uncertain of its precise nature, the mycelium is undoubtedly the tissue invasive phase and at first incites an acute polymorphonuclear leucocyte reaction in its vicinity Surprisingly little evidence of necrosis is shown by closely adjacent tubular epithelium or in nerve cells adjacent to cerebral lesions (Pl. 1 fig 4).

In the first few days of infection the organism is widely disseminated by the blood stream and can be found by careful histological and mycological examination of many organs and by blood culture. That the organism is present in blood vessels in most tissues, and is furthermore viable, can be shown by culturing organ slices, after rinsing in ethanol and a solution of

antibiotic, on Sabouraud's medium for 2 days. The preparation is then examined histologically (Pl. 1 fig 5). Blood vessels are found to be packed with blastospores which were not visible in histological preparations made of uncultured slices. Lesions at this early stage are, however confined to those organs with an abundant blood supply namely brain, heart, muscle and kidney in all of these blastospores can be seen in capillaries with mycelium extending into the surrounding tissues (Pl. 1 fig 6). Lesions are not found at this time in the spleen though many yeasts may be found in splenic macrophages in the later stages of candidal infection in rabbit, mouse, rat and guinea pig occasionally tiny granulomata may also be found. Similarly lesions are scanty in liver lymph nodes and lungs, all being seats of abundant reticuloendothelial cells. *Candida albicans* is not a potent killer of cells and provided that sufficient macrophages are available it will, ultimately be engulfed.

Rabbits surviving for several weeks, following intravenous injection of *Candida albicans* show granulomatous lesions in brain, heart, kidneys and other organs (Pl. 1 fig 7) and the parasite is often difficult to find in such lesions It is, however occasionally seen in small numbers at the periphery of such granulomata. The importance of histiocytes in the elimination of candida from the tissues has recently been emphasized by Mankoff & Adam (1957). In fact the host is extraordinarily well adapted to deal with moderate numbers of those organisms An even more vigorous cellular response to candida can be obtained in those animals which have recovered from a small dose of living *Candida albicans* and some days later are given a larger lethal dose. Granulomatous lesions with necrosis and giant cell formation (Pl. 2 fig 8) now become evident in liver and spleen though the organism is again often difficult to find this seems to be a hypersensitivity response.

THE MYCELIAL PHASE OF *CANDIDA*

Candida albicans produces abundant mycelium in rabbit, guinea-pig, rat and mouse tissues though the latter two animals are less susceptible to it than the former two. No other candida that we have used produces such abundant mycelium in tissues *Candida tropicalis* produces an extensive mycelial outgrowth in culture (Pl. 2 fig 9), but not abundantly in the tissues of the animals which we have studied (Pl. 2, fig 10). The *in vitro* mycelium of *Candida tropicalis* is rapidly formed, but as rapidly degenerates cells become bloated and irregular in shape and size. Such a rapid involution of the mycelial phase of *Candida tropicalis* may explain its lesser pathogenicity as compared with *Candida albicans*.

The mycelium produced by *Candida albicans* may provide an explana-

PLATE I

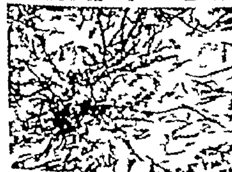


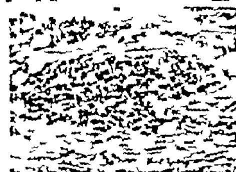












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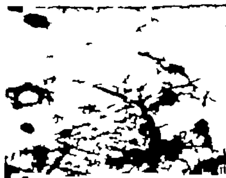
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PLATE 2

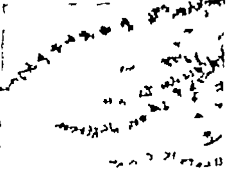














For explanation see p. 81

tion of its universal pathogenicity in the animals that have been investigated. In brain smears from rabbit lesions it resembles the penetrating structures seen in dermatophytes invading keratin (Davidson & Gregory 1934). The mycelial phase is not to be considered a degenerative one; rather it is more specialized than the blastospore phase. It enables fungal protoplasm to penetrate into the tissues as a continuous syncytium with a large absorbent surface.

PATHOGENICITY OF *CANDIDA*

Repeated examination of smears and impression preparations from recent candidal lesions have shown that the mycelial form of *Candida albicans* is capsulated (Pl. 2, fig. 11). This capsule has been noted before and studied biochemically (Negroni, 1936*a*). It may be the source of the endotoxin postulated by Henrici (1940) and other later workers (Salvin, 1952; Winner, 1958). It compares well with the infection hypha of plant parasites such as wheat rust, *Puccinia graminis*. This infection hypha is covered with a mucilaginous envelope in the vicinity of which the parasitized plant cell wall shows chemical changes.

The lesions produced by *Candida albicans* in guinea-pig, rat and mouse are similar in type and distribution to those which have been described in the rabbit. Death from uraemia following massive doses of *Candida albicans* seems to be due in part to blockage of renal capillaries, by masses of blastospores and mycelial networks. This is supported by a progressive diminution of renal plasma flow and the development of oliguria in rabbits dying acutely of uraemia following intravenous injection of *Candida albicans*. The renal plasma flow was determined by measuring the clearance of a single intravenous loading dose of para amino hippuric acid (Gresham & Davidson, 1959). It is probable that intravascular mycelial formation or some other factor plays a part in vascular obstruction by *Candida albicans*, since intravenous injections of comparable doses of organisms of similar size, namely *Candida parapsilosis* and *Candida guilliermondii*, produce no such effect nor can the same effect be produced by intravenous inoculation of a formalin-killed suspension of *Candida albicans*. It has likewise been shown in the rabbit and other species that glass spheres larger in diameter than glomerular capillaries (50–180 μ) could be recovered from the renal vein following injection into the renal arteries (Simkin, Bergman, Silver & Prinzmetal, 1948). This again casts some doubt upon a purely mechanical obstruction by blastospores alone and raises the possibilities of mycelium and thrombi, the latter probably produced by some endothelial toxin, as other factors in causing vascular blockage.

SPREAD OF CANDIDAL INFECTION

In those animals that survive the initial renal insult lesions develop in other organs. These may be due to blastospores introduced at the primary inoculation or a secondary blood invasion, via vascular shunts, from the primary renal lesion. Histological examination of tissues at this stage will show mycelial elements in cerebral capillaries (Pl. 2, fig. 12) and the blood stream. The mycelial phase is then also of importance in blood dissemination as well as in tissue invasion. This view accords with the finding of circulating hyphae in experimental dermatophyte infections (Simons, 1954). Experimental infections, then, support the view that the mycelial is the most efficient pathogenic form and the blastospore an artificial form produced by culture in sugary media. This is further confirmed by the incubation of kidney slices taken from rabbits dying with *Candida albicans* infection. Before incubation kidney sections reveal abundant mycelium after 24 hr. incubation in 2 per cent glucose broth at 37° C. most of the mycelium has been replaced by proliferating masses of budding blastospores. Guilliermond in 1909 regarded the yeasts as degenerative forms of mycelial fungi. Dimorphism in the genera histoplasma and blastomyces is in a reverse direction, the blastospore form is the parasitic one and it is probably by analogy with such organisms that the fallacy of loss of pathogenicity in the mycelial phase of candida has evolved (Scherr & Weaver 1953). American studies of neonatal thrush have shown that two distinct stages exist before the lesions appear. Blastospores appear first, then mycelium a day or so before the lesions are clinically evident (Rogers, 1957); they consider that the appearance of mycelium in lesions may be a pathognomonic feature of *Candida albicans* infection.

CANDIDA TROPICALIS

Despite statements to the contrary (Adriano & Schwartz, 1955) *Candida tropicalis* is a pathogenic organism for laboratory animals. Its mode of action is largely an obstructive one in the kidneys and it is lethal for rats and mice; it only produces a transient rise of blood urea in rabbit and guinea-pig a few days after intravenous inoculation. The fungus produces abundant mycelium in culture (Pl. 2, fig. 13) but does so less often in the tissues. Histological lesions in rat and mouse reveal abundant blastospores blocking blood vessels with little mycelium formation. Masses of blastospores may be found in renal pelvis, ureters or bladder and sometimes lead to an obstructive hydronephrosis (Pl. 2, fig. 14); balls of *Candida tropicalis* may be found within the urinary bladder. Death is due to uraemia in those

animals dying within a day or so of intravenous inoculation of *Candida tropicalis* and many vessels are plugged with blastospores. If the animal survives, blastospores pass into the nephron and down to the renal pelvis producing strands of chronic purulent inflammatory infiltrate containing many blastospores but only a small amount of mycelium.

The amount of mycelium produced by *Candida tropicalis* is scanty though more is seen in rat and mouse tissues than in the experimental infections in guinea-pig and rabbit. Vascular blockage is again probably the factor causing uraemia in *Candida tropicalis* infections in rat and mouse though mycelial formation cannot be the only explanation of such blockage since it is so scanty. Again the possibility of a toxin arises: the finding of a capsule around short hyphae and blastospores, in smears from lesions, suggests that here again, as in *Candida albicans* a capsular substance may be the aggressin.

Intravenous inoculation of *Candida tropicalis* suspended in 2·5 per cent gastric mucin leads to a greatly increased number of lesions in the mouse kidney. This may be due to an increased tendency for vascular blockage or due to the provision of a more aggressive surface. The use of mucin to enhance the pathogenicity of micro-organisms was proposed by Salvin, Cory & Berg (1952). Intravenous inoculation of *Candida guilliermondi* or *parapsilosis* suspended in such a medium leads to the production of small myocardial and hepatic lesions, containing blastospores. Without mucin no lesions at all are produced. We have not been able to produce lesions in any of the animals studied with either *Candida guilliermondi* or *parapsilosis* unless they be suspended in gastric mucin. Nevertheless culture of organ slices made a week or more after intravenous inoculation of such organisms will reveal clumps of blastospores in vessels when such slices are examined histologically. It has also been possible to recover *Candida guilliermondi* from the peritoneal cavity of mice as long as one week after intraperitoneal inoculation. It seems, then, that these organisms are much less able to proliferate *in vivo* than *Candida albicans* and *Candida tropicalis*. This cannot be explained merely by lack of an aggressin nor by a failure to form mycelium. Both *Candida guilliermondi* and *Candida parapsilosis* are dysgonic in culture: both grow slowly and the latter also produces a rough dry colony. It may be that these growth characters prevent the accumulation of an infective dose in tissues since reproduction cannot keep pace with phagocytosis.

CONCLUSION

In this brief account of the experimental pathology of candidal infections we have mentioned some of the factors concerned in determining fungal saprophytism and parasitism. The species which we have studied cover the spectrum depicted in Table 1 At one extreme we have the universal pathogen *Candida albicans* it is capsulated in tissues, probably producing a toxin and invading by extensive mycelial formation. At the other extreme *Candida guilliermondi* and *parapsilosis never cause lesions in experimental* animals unless aided by mucin nor do they form a stable mycelium in culture or tissues. One exception to this statement is the mycelial form of *Candida guilliermondi* in experimental human nail fold infections (Whittle & Gresham, 1959). Here the mycelial phase colonized the fold but did not produce clinical evidence of disease.

The final answer to the problem of cellular damage by micro-organisms depends upon an understanding of the chemical interaction between cell and micro-organism. A convergent attack from bacteriological, mycological, virological and histopathological aspects should achieve that understanding more rapidly than any concentrated attack on one small facet of the problem. The need for a broad biological approach, particularly in the training of medical men and the pursuit of medical research, is a pressing one today Perhaps the value of such an approach is evident from our discourse G A G

APPENDIX A

Taxonomic characters of the various species of candida were investigated as soon as possible after isolation in pure culture from an infected animal usually within 40 hr after primary isolation on Sabouraud s medium.

The formation of hyphae, growth of colonies, and fermentation of sugars was found to be so speeded that complete results, with the exception of chlamydospore formation, were available in 12–18 hr rather than 24 hr or longer Checking for purity was of course maintained, and results were repeatable.

For inoculations of diagnostic media turbid suspensions (BT 10) of the yeasts in physiological saline was used. From a Pasteur pipette one or two *drops* were distributed into each sugar tube. For sol'd media, one light touch from a small loop was sufficient to start a giant colony growing and a straight wire stab, streak or smear was used for slants or slide culture. The bungs of the sugar fermentations were pushed down and sealed with wax—most easily by Pasteur pipette again—while those of broths, etc., from which subsequent samples had to be taken were not sealed. Previous tests had ascertained that a wax seal and a 2 per cent sugar solution were best for fermentation and collection of gas though as in all things mycological, by a relatively narrow margin. Incubation in a candle CO_2 jar made no appreciable difference to the colony and slide growths, though it did inhibit the biochemical reactions a little

Table I

Name	Sabouraud agar	Sabouraud broth	Corn meal agar	Potato water	Glucose	Sucrose	Maltose	Lactose	Remarks
Candida albicans	Rich cream-coloured colonies, becoming rough in centre, [illegible] folded edges. Domestic yeast smell	Clear supernatant moderately heavy, sometimes membranous sediment	*Colony* Dull porcelain hue. *Stab.* Bottle-brush growth mycelium with blastospores, clusters and in balls, and chlamydospores	Strongly mycelial strains can produce [illegible] than characteristic various through to non-mycelial membranous growth of blastospores, and is constant per strain. Chlamydospores are produced. *Morphology* Round blastospores, singly or in small [illegible] aggregations, elongated cells, short pseudohyphae, long thick pseudohyphae, and thinner extensive mycelium	AG	A	AG	—	Sucrose fermentation is constant variable cellular and mycelial degeneration is least pronounced of all the candida, and the morphology the most orderly
Candida tropicalis	Rapidly growing large colony cream coloured to greyish dun. Rough, high with radiating mycelial processes. Entering the agar and composed of hyphae with thick clusters of blastospores	Marked gas production, surface film, slightly turbid broth, thick sediment. Heavy sour yeast smell	*Colony* Porcelain white, rough centre, carpets of mycelium with blastospores	Surface film, few flecks in the broth, thick membranous sediment. *Morphology* Large ovoid cells, budding, elongating mycelium with blastospores. Cells sometimes bottle-like; rapid degeneration	AG	AG	AG	—	The biochemical and morphological reactions are perhaps the most stable of the candida, but the cellular and mycelial degeneration is such that though large volume of growth is produced, relatively little of [illegible] is viable
Candida parapsilosis	Small, folded porcelain hue colony [illegible] with even turbidity and particles	Particulate growth floating in supernatant fluid and adhering to glass. Fine sediment	*Stab* Thin knotted cord growth, mycelium meagre. *Colony* Knobby small, shiny colonies	Growth in media as in Sabouraud broth. *Morphology* Small, granular, rather ellipsoidal cells, tending to become irregularly wedge shaped. Short pseudohyphae, sausage-like chains	AG	A (—)	A	—	There appear to be [illegible] number of rough wild candida, sometimes pigmented, resembling *C. parapsilosis* and usually forming surface film. The necessity of watching for rough forms of yeasts is apparent
Candida guilliermondii	Fine, [illegible] shiny mother-of-pearl colonies bluish surface tinge and dun to dark coloured back of colony	Clear supernatant, fine sandy sediment	*Stab* Thin, knotted cord growth, mycelium meagre. *Colony* Fine, white	Growth of media as in Sabouraud broth. *Morphology* Small, granular rather lemon-shaped cells, elongating to slipper-like cells, and short striated pseudohyphae fragments. Tendency to clumping and formation of giant cells	$\overline{AG}$	$\overline{AG}$	—	—	This organism appears characterised by delicacy of growth and appearance. Its reactions are [illegible] but slower than the preceding candida
Saccharomyces cerevisiae (species from cheese)	White, high domed rough friable colonies, spreading to rich orange with production of strong odour of cheese	Thick surface growth, slightly turbid supernatant, thick sediment	*Stab* Fine, granular cord growth mycelium with age. *Colony* Dull, translucent, paste-like, with star radiating processes in several days	Growth in media as in Sabouraud broth. *Morphology* Cells long, oval, rapidly degenerate. Aggregations and chains of cells form radiating processes; mycelium with age	A	A	A	A	The mycelium appears when the growth is old and in phase of relative degeneracy. [Reference book "The Yeasts. Lodder, J. & Van Rij, K. 1952. Amsterdam.]

A = acid; G = gas formation; V = weak acid formation.

For any plates which are to be kept some time it is advisable to seal them off, or have some device to cut down fungal contamination. The broths to be examined for growth of mycelium and typical patterns of surface film and sedimentation naturally must not be agitated. But it is essential to shake the sugar tests periodically to free the bottom of the Durham tube from growth, or the gas will escape uncollected, despite the wax seal and the use of small volumes (1·5 ml.) of the sugar solution. The other factors in examinations are media, and the diagnostic reference work used. There are some standard rather simple media —such as Sabouraud's and maltose corn meal agar—which provide a base for the rest of the reactions so many varieties of media have been used successfully and it matters little what media be used provided that they are tested out with known yeasts from recognized stock cultures. All variations necessary to stimulate thought and caution are taken care of by these micro-organisms themselves. The sugar reactions given below are very brief further tests involve the working out of an organism by means of galactose, raffinose mannitol, and arabinose—to name a few sugars and the use of the Auxanogram technique which is very valuable in practised hands. Such methods were not carried out in this series of experiments where the organisms were known. Variation in biochemical observations appears to be a factor in mycology from simple short sugar tests to gas tensions and protein reactions.

In the matter of reference texts, the case of *Candida guilliermondi* can be cited in one it is stated to ferment glucose, galactose, lactose, sucrose and maltose with the production of acid only throughout and in another to be entirely non fermentative. It is suggested that only one digest of biochemical reaction tables compiled in a personal laboratory manual or one mycological laboratory manual be used, in a given period. Then, it would seem reasonable that the media used and the reference author's media should agree in essentials, particularly in respect to exotic ingredients—not as fatuous advice as it seems.

With these few elementary rules the reactions of the candida used in the foregoing experimental series are presented. M B

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EXPLANATION OF PLATES

PLA

Fig. Bisected kidney of rabbit killed days after LV *Candida albicans*

Fig. 2. Mycelium in vessels in the kidney of rabbit. P.A.S. ×450.

Fig. 3 Impression preparation of candidal lesion in the rabbit kidney Gram ×450.

Fig 4. Brain lesion in rabbit killed 3 days after I.V *C albicans*. Note mycelium, scanty leucocytic reaction and well preserved neurones. P.A.S. 450.

Fig. 5. Blastospores in an hepatic vessel in section of incubated liver from rabbit infected with *C albicans*. P.A.S 5.

Fig. 6. Mycelium of *C albicans* spreading from cluster of blastospores in myocardial capillary P.A.S ×450.

Fig. 7 Granuloma in rabbit skeletal muscle following repeated injections of small doses of *C albicans*. P.A S. 350.

PLA

Fig. 8. Giant cell with sparse collection of macrophages in rabbit liver following repeated small injections of *C albicans*. P.A.S. 300.

Fig. 9. *Giant colony of C tropicalis on corn-meal agar*

Fig o. Blastospores of *C tropicalis* in Bowman capsule of mouse given the organism I V Note the absence of mycelium. P.A.S. 450.

Fig Impression preparation of cerebral lesion in the rabbit due to *C albicans*. Note the capsule. Gram. 50.

Fig. 2. Mycelial threads of *C albicans* in retinal capillaries. P.A.S. 450.

Fig. 3 Slide culture of *C tropicalis*.

Fig. 14. Hydronephrosis in the mouse due to renal pelvic obstruction by *C tropicalis*.

THE BACTERIAL CELL

By E. F. GALE

The material on which Dr Gale's lecture was based is to be found in the two published Symposia of the Society for General Microbiology entitled *Bacterial Anatomy* (1956) *The Strategy of Chemotherapy* (1957). Some of the material is also available in a more condensed form in Dr Gale's Leeuwenhoek Lecture, 'The biochemical organization of the bacterial cell' *Proc Roy Soc.* B 146 (1957)

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PSYCHOPHYSIOLOGICAL MECHANISMS

PSYCHOLOGICAL MECHANISMS IN PSYCHOSOMATIC DISORDERS

By D RUSSELL DAVIS

My discussion of the psychological mechanisms centres on one frequent although not invariable feature of psychosomatic disorders the stereotyping of the reaction.

Stereotyping is seen in its most obvious form in the disorders which recur in attacks, such as migraine, asthma, peptic ulcer ulcerative colitis and atopic dermatitis. Each attack tends to be just like the previous one, the same set of symptoms appearing in the same sequence. The pattern of the attacks, and the underlying physiological changes, tends to show a remarkable consistency even when the attacks are spread over years or decades, although it may undergo gradual modification. Each patient has his own characteristic and peculiar pattern. Its consistency is not an artifact of his description, for it is also displayed in physical signs.

Within certain limits, the pattern remains the same although the stressors which evoke the reaction vary This consistency despite changes in the stressors is perhaps shown most clearly in children of school age. Some children suffer from bilious attacks or stomach upsets, for instance, whenever there are troubles, and whatever more or less the character of the trouble. Others suffer from headaches, and others from bowel upsets, upper respiratory tract disorders or eye-strain. Similarly in adult patients anxiety attacks, for instance, of a stereotyped pattern may be evoked in a variety of stressful situations. The reactions are specific and stereotyped, the stressors more or less diverse. However there are some cases in which an anxiety attack is evoked only by specific stimuli falling within a narrow range of meaning An extreme example of specificity in the stressor although another kind of stressor is provided by those allergic reactions which are evoked only by proteins belonging to a narrow and special class.

In many cases the pattern of a psychosomatic reaction appears to be established during early childhood. This is often so with atopic dermatitis, the pattern of a first attack occurring, say in infancy being reproduced in site, form and course with little significant modification in recurrences for two decades or more. The patterns of asthma and migraine attacks tend also to be established relatively early in life. But the first of a series of stereotyped reactions may appear at any age. In some cases the pattern which becomes established has been adumbrated in previous illnesses.

Consistency in the reactions of individuals to stressors has been demonstrated experimentally notably by Lacey and his collaborators (1952, 1953), who have emphasized that the strain is always distributed unevenly there is over-action of some mechanisms and under-action of others. Experimental stressors such as the cold pressor test or insoluble problems of various kinds, evoke measurable reactions in cardio-vascular functions, skin conductance, etc. From observations on these functions a profile can be drawn for each subject, which shows the pattern of the reaction of his autonomic nervous system. This profile tends to be reproduced on retesting when the stressor is the same, and again when the stressor is different. The maximal activation tends to occur in the same physiological function. *The organ emphasis* as it has been called, is in a considerable degree independent of the character of the stressor There are of course differences between individuals in the degree of consistency shown in series of tests. Some individuals show a remarkable specialization and stereotyping Lacey has asserted, on the basis of experimental evidence, that specialization and stereotyping in the reactions of the autonomic nervous system to stressors occur at least as early as six years old.

VARIABILITY IN REACTIONS

To emphasize the stereotyping of psychosomatic reactions is not to deny that there also occurs some variability A patient, for instance, who has suffered from one or more attacks of atopic dermatitis may develop asthma or perhaps, later on in life, rheumatoid arthritis. Another patient who has suffered for years from migraine may develop ulcerative colitis. The peptic ulcer patient may develop hypertension. In other cases the salient symptom may remain the same, but the pattern of the attack changes. Again, the history often shows variety in the illnesses from which the patient has suffered. A patient, for instance, suffering from a psychosis gives a history of migraine, alopecia areata and atopic dermatitis. Although there is variety in such cases, there is also stereotyping

Again, at the physiological level of description the pattern of the reaction, for example, that of the adrenal medulla, varies with the character of the stressor situation. Thus it has been shown that in anger in situations in which the expression of aggression is prevented, and anxiety is the prevailing emotion the excretion of adrenaline in the urine shows a much greater increase than does that of nor-adrenaline. The opposite is the case in anger-out situations in which there are outlets for aggression, and anger is felt, for there is then excessive excretion of nor adrenaline (for example Funkenstein King & Drolette, 1957). Similarly as Selye (1946) has shown,

the pattern of the stress responses composing the general adaptation syndrome varies with the conditions that are established experimentally The extreme view is that of Alexander (1950), who has insisted that every emotional stimulus has its own physiological syndrome It is remarkable how much this last view has to be qualified.

The experimental evidence which shows how the pattern of the physiological reaction varies with the character of the stressor would lead one to expect correlations between the patterns of reaction observed clinically and types of stressor situation, or classes of conflict or life-experience Moreover it is in the tradition of medicine to explain differences in the form of the disease by reference to differences in the pathogenic agent. Attempts to explain psychosomatic reactions similarly have had a very limited success. Many efforts have been made to demonstrate correlations. H. G Wolff and his collaborators (e.g Schottstaedt, Grace & Wolff 1956), in particular have emphasized the variations in somatic responses with different kinds of conflict situation. Patterns of reaction and stressor situations have been classified in various ways, the former according to the organ emphasis for instance, and the latter according to whether they affect the individual in the sphere of occupation, social role or sexual relationships, for instance. The results have been slight. Within each class of reaction it has been usual to find a diversity of stressor situations, and there have been few consistent differences between one class and another

There are a few positive suggestions in the literature. Peptic ulceration has been reported by Davies & Wilson (1937) to occur typically as a result of work or financial difficulties or family misfortune, whereas atopic dermatitis in adults has been said by Kepecs and his collaborators (1951) to occur in relation to conflicts in the sphere of hetero-sexual relationships. However comparison between the two samples is hardly possible, not least because there are also large differences between them in the distributions between the two sexes, age groups and social-class groups.

It is of course possible that the relative lack of success attending attempts to correlate patterns of reaction and stressor situations has been due to the inadequacy of the methods of assessing and classifying the variables. It can still be maintained, therefore, that specific factors in the situations exist although they have not yet been identified, and that positive correlations will be found when the stressors are classified appropriately More sophisticated hypotheses are required, as Weisman (1956) amongst others has shown.

STEREOTYPING IN BEHAVIOUR

It is also a possibility that reactions tend to be stereotyped because the stressors which affect individuals tend to be more or less constant. Those who suffer from recurrent psychosomatic disorders tend to be of neurotic personality broadly speaking, and to be prone to run into difficulties of particular kinds. Thus they tend to be passive, dependent, unassertive and insecure in particular they tend to have difficulty in expressing their aggressive feelings. Peptic ulcer patients, for instance, have been described as always in the front-line of life's activities. They tend to be conscientious and moderately successful in their professions or occupations, but because they are ambitious they are also dissatisfied. Because of these qualities they expose themselves to affronts and disappointments in their work.

It is a common-place finding in psychiatric practice that neurotic individuals run over and over again into the same kind of difficulties in their dealings with others, and there is often a monotonous uniformity in the stressful situations which they appear to create for themselves. They go on making the same mistakes. Furthermore, important characteristics of much of the behaviour observed in neurosis are repetitiveness and resistance to modification and extinction, although the behaviour is poorly adaptive. Thus stereotyping is commonly a feature of disorders of behaviour and it poses theoretical problems of great interest and importance (Davis, 1957 p 273). One might argue that stereotyping in somatic reaction is an in direct consequence of stereotyping in behaviour but it is probably better to regard them both as parallel manifestations of the same general tendency

PERSONALITY AND TYPE OF DISORDER

It is widely supposed that sufferers from recurrent psychosomatic disorders tend to be of neurotic personality although the evidence is inconclusive. There appear to be broad similarities, therefore, in the personalities of patients falling into different clinical groups. Probably there are also differences, but so far these are ill-defined and systematic studies have not yet provided any convincing evidence that they exist. The methods at present available for studies of this kind are still far from satisfactory. In every clinical group there is certainly some diversity in the personalities of its members, although one or more types of personality may appear to be unduly common.

THE PSYCHOSOMATIC APPROACH

Stereotyping characterizes attacks of migraine, asthma, peptic ulcer ulcerative colitis and atopic dermatitis. These examples read like a short select list of typical psychosomatic disorders. But to be representative of psychosomatic disorders this list would have to be extended. Keeping its diversity one might add diabetes mellitus— When stocks go down in New York, diabetes goes up as Crile remarked thirty years ago—essential hypertension, rheumatoid arthritis, carcinoma of the breast and so on In these disorders stereotyping is much less apparent, if it occurs at all. The list could be very greatly extended, for the evidence suggests that there are few disorders in which psychological factors do not play some part in the aetiology Admittedly inclusion in the list is controversial in every case.

It is doubtful whether a distinction between disorders which are psychosomatic and those which are not has now any practical or theoretical value. To make a distinction of this kind anyhow misses the essential point of the new attitudes, or perhaps the old attitudes revived, which make up the so-called psychosomatic approach. To describe a disorder as psychosomatic is to recognize that in some way psychological factors play a significant part in the aetiology in conjunction with other factors. It means that the aetiology is multiple, and that psychological as well as somatic mechanisms are taken into account. The important questions lie not in whether psychological factors play a part, but in the definition of the mechanisms through which they work.

Psychological factors are of more practical importance in some disorders than in others. They may be regarded as more important in urticaria and angina pectoris than in leukaemia, although even in leukaemia they are not wholly without importance (for example, Greene & Miller 1958) Even when psychological factors have obvious importance in the aetiology as in the former two disorders, drugs or other methods of treatment may be as effective as psychological methods. It is tempting to draw the conclusion that disorders in which stereotyping is marked are those in which psychological factors are of particular importance. Anxiety attacks, for instance, tend to show marked stereotyping But such a conclusion would be dubious, for stereotyping is also shown in marked degree by allergic reactions due to protein sensitivity in which psychological factors may or may not be of importance. It is also shown in epilepsy even when it occurs as a symptom of an organic lesion. The aetiology of the disorders showing stereotyping appears to be varied in this respect.

Stereotyping when it occurs is far from complete, and the pattern of reactions varies in some degree in correspondence with variations in the

stressors. Yet it is sufficiently common and sufficiently marked to be regarded as an important feature of many recurrent disorders. How does it come about?

THE ROLE OF INHERITANCE

The popular idea is that everyone has his Achilles heel, and that the effects of strain are shown at the point of his special weakness. In other words, each individual has a weak or vulnerable organ, which is the first to succumb whenever he meets stressors of above a certain threshold of intensity and in some degree regardless of their character

It is a short step to go on to suppose that the weakness is constitutional and inherited. The familial tendency in the incidence of many recurrent disorders and the appearance of stereotyped patterns relatively early in life are compatible with this view but do not decide in its favour against a hypothesis of environmental determination. The pattern of incidence within families of particular forms of reaction has not been shown to conform with that expected on any specific genetical hypothesis, and it seems unlikely that inheritance alone accounts for the weaknesses, although no doubt it contributes towards them. However little further can be done to decide the importance of genetical factors until the nature of the weaknesses has been given clearer definition.

It used to be confidently assumed that the pattern reactions are inborn, and even so extreme an environmentalist as J B Watson (1919) discussed them under the rubric Hereditary modes of response. In support of this view he remarked that the separate details of response appear with some constancy with some regularity and in approximately the same sequential order each time the existing stimuli are presented (p. 195). It seems necessary now to depart from this traditional view and to consider how far the patterns are the product of experience.

THE ACQUISITION OF REACTION PATTERNS THROUGH LEARNING

If a reaction pattern is not fully determined by the constitution of the organism, then it is acquired as a result of what the organism goes through, that is its experience of its environment. I use here the term experience deliberately in a broader sense than is usual to cover not only psychological but also physical and biological events, for these affect behaviour just as much as psychological events do although the mechanisms through which they work may be very different. The habits or reaction patterns displayed by the organism at any given time are the product of its constitution and experience, physical, biological and psychological.

In some cases the history suggests that a stereotyped reaction first appeared after an illness with a specific aetiology such as trauma or infection, and affecting a particular organ. Thereafter the organ has shown a special vulnerability Whooping cough, bronchitis and then asthma is an example of such a history In other cases the first event has been an event of psychological significance—the birth of a sib for instance, especially when the patient has been over protected In other cases no first event can be identified. Commonly the onset of a disorder in an adult patient has been preceded by an event such as an operation, childbirth the menopause, natural or artificial, an infection or the death or illness of a relative or friend. In many cases there has been a combination of two or more such events. But it seems probable that in most cases experiences of all three kinds co-operate to determine the pattern of the somatic reaction. However the discussion below is concerned mainly with the acquisition of reaction patterns through the processes of learning

We know something about the course of development in childhood of the habits served by the central nervous system—motor perceptual and intellectual skills and so on—although we know little about the factors responsible for distortion or retardation in their development. Although physical maturation may play some part, these habits and skills are acquired largely through learning, the course of which is from diffuse undifferentiated activity to more organized and specialized responses. As learning proceeds, responses become more stable and more regular in fact more stereotyped. As individuals grow older their habits tend to become set and idiosyncratic. They bring more and more specialized equipment to bear upon the tasks which face them in their everyday living.

In the initial stages of learning the organism shows trial-and-error activity The unsuccessful components drop out. The successful components gain ascendancy This process of elimination and selection, which constitutes learning, is governed by what is now usually known as the principle of reinforcement This states that the tendency to make a response is reinforced if it achieves the satisfaction of a need the tendency to make responses which fail to achieve satisfaction is weakened. That is, responses are reinforced *or weakened according to their effects. The repetition of a* response in association with a stimulus or another response does not bring about learning unless the effect is to satisfy a need.

It seems reasonable to expect that the course of development of habits served by the autonomic nervous system and other systems not under voluntary control is similar Our knowledge is very scanty Individual differences in reactions of the autonomic nervous system can be demonstrated within the first few days of life (Grossman & Greenberg, 1957), but

it is not known whether they have any consistency or whether they persist. The pattern varies with the stressor and it is possible to distinguish patterns which correspond to fear love and rage, as Watson did, although a different classification may be preferred. By three months old reactions show a degree of consistency each infant already having distinctive characteristics. There is anecdotal evidence (for example, Seitz, 1950) to show that psycho-cutaneous reactions can be learnt as early as the second week of life. The so-called emotional reactions in early infancy are diffuse and undifferentiated, but become increasingly differentiated and specialized during the first year and thereafter (Bridges, 1932) On such evidence as is available it seems probable that fairly stable idiosyncratic reaction patterns are established by the time the child is six years old.

A few reports in the literature suggest that the patterns are influenced to a considerable degree by the amount and character of the attention the child receives from his mother At any rate it seems worth while to look for some of the relevant variables in the mother-child relationship, although factors of other kinds have also to be taken into account. Investigators have paid less attention however to patterns of autonomic-nervous-system reactions than to such aspects of behaviour as crying

In the first attack of a recurrent disorder such as asthma or atopic dermatitis, the somatic symptom is commonly accompanied by multiple anxiety symptoms and appears as but one component of a relatively diffuse reaction. In later attacks it has gained ascendancy and there tends to be little or no accompaniment of anxiety symptoms—indeed sometimes surprisingly little. Whereas the majority of normal persons show relatively diffuse emotional expression in reaction to frustrations and disappointments, the patient prone to asthma develops an attack—nothing else. This, incidentally is the seeming paradox to which critics of the psychosomatic approach occasionally refer If asthma is due to emotional disturbance, why then is there no emotional disturbance? they ask, using the term emotional disturbance in two different senses.

Commonly the history of a first attack of a disorder is as follows the patient suffers a disappointment or bereavement multiple anxiety symptoms appear a somatic symptom emerges the anxiety symptoms gradually subside and the somatic symptom becomes increasingly prominent. This sequence, one may argue, parallels trial and-error learning and invites explanation, therefore, by reference to the principle of reinforcement. Admittedly a history of this kind is likely to be in some degree an artifact, for the patient's encounters with doctors are likely to encourage him to focus his attention upon the somatic symptom and to place the emphasis on it in his accounts of himself Accordingly he may give less prominence

to feelings of hopelessness and dejection than they warrant. However it is probably true that as they persist or recur reactions tend to become increasingly specialized and idiosyncratic.

There has been keen controversy amongst psychologists in recent years on the question whether reactions served by the autonomic nervous system are learned in accordance with the principle of reinforcement, or whether it is necessary to suppose that their learning is governed by the law of association (for example, Mowrer 1950 Osgood, 1953) There is one obvious difficulty in the application of the principle of reinforcement. Reactions served by the autonomic nervous system are anticipatory and not consummatory it would appear and do not achieve satisfactions. They appear to add to rather than take away from the physiological disturbances which constitute needs.

It is well known that reactions served by the autonomic nervous system can be conditioned In fact, all Pavlov's early work on conditioning was based on the salivary reflex, and his theory was an elaboration of the law of association. But the results of more recent experiments suggest that conditioning does not occur unless the evocation of the unconditioned reflex by the conditioned stimulus brings with it some need reduction directly or indirectly The pupillary reaction to light has been a crucial instance. Earlier experiments showed that the reaction could be conditioned so that it was evoked by verbal commands, but recent attempts in more refined circumstances which have excluded need reduction have been unsuccessful (Hilgard, 1956) The psychogalvanic reaction is readily conditioned, but probably only in circumstances in which there is also need reduction. The crossed vaso-constrictor response induced in one hand when the other is immersed in ice-cold water has also been conditioned, it is reported, but the experiments do not allow any conclusion on the part played by need reduction.

In patients, local reactions commonly increase in intensity as time passes, in such a way as to suggest that repetition increases the tendency to react. Kuno (1956), for instance, puts forward this explanation to account for the gradual increase in the local sweating in emotional disorders. He thus depends on an association theory of learning

However most authorities are loathe to accept, as Mowrer does, that the learning of reactions served by the autonomic nervous system is governed by a different principle from that which governs the learning of reactions under voluntary control. Yet to suppose that it is governed by the principle of reinforcement and to abandon the law of association, raises the question what effects reinforce the reactions? This question is a crucial one in contemporary research on the psychological mechanisms concerned

in psychosomatic reactions. If it can be answered, it will be possible to go on to answer the further questions why do the reactions persist and recur when some at least of their effects are maladaptive? And how can they be extinguished? Attempts to answer these questions require more precise hypotheses about the nature of the effects and the mechanisms of reinforcement than those current at present.

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PSYCHOLOGICAL MECHANISMS IN THE PSYCHOSOMATIC SKIN AFFECTIONS

By J. H. CULLEN

The notion that psychological factors have an important role in skin affections is not at all a new one. In 1856, Erasmus Wilson in his book *Diseases of the Skin* claimed that amongst the causes of eczema were affections of the nervous system, as mental emotions particularly of the depressing kind. The century intervening between then and our own days has seen an inevitable trend towards specialization which has accompanied the great advances in scientific medicine.

Rational medicine must, of course, include some kind of holistic concept of the organism because, without it, no account of any disease process can be adequately explained. The Cartesian dualistic position, however, has so permeated our everyday thinking that we find it difficult to prevent its determining our attitudes to scientific problems. This is not to deny that there have been frequent pleas for a reassertion of the role in medicine of the concept of the human organism as a psychophysiological unit. From the point of view of biological science disease is a reaction between the whole organism and its environment. I want to stress this general concept of disease as a maladaptation between the organism and its environment because the term which is usually applied to accounts of disease which include psychological factors—psychosomatic—has unfortunate implications of a body mind dichotomy. Modern psychological theory allows of no such dichotomy, although for purposes of observation and description within our present language structure the two aspects of the organism may be distinguished.

The application of such a concept of adaptive processes to diseases of the skin would seem, perhaps, an obvious step because the skin, which is one of the largest of the body's organs, forms a mediating barrier between the organism, with its relatively stable internal environment, and the unstable external environment. But by contrast with disorders in other organs the skin diseases present problems of very great complexity and no fully satisfactory picture of the processes which become disordered is as yet available to us. The position in psychopathology is even less satisfactory and theory far outdistances experimental evidence. What is demanded, if an adequate account of the role of psychological mechanisms in the psychosomatic skin affections is to be rendered, is the bringing together of the

most general theories in psychopathology which are applicable to the adaptive behaviour of the whole organism with the concepts we have of the mechanisms by which the functions of the skin become disordered and produce symptoms.

I cannot hope, in this paper even to begin to show how some of these difficulties can be resolved. At most I can only select some of the processes and reaction patterns of the skin and show how they may be studied with reference to the role of emotional factors in their behaviour

Perhaps the simplest and yet one of the most important of the mechanisms involved in the majority of cases is the cutaneous vasomotor activity Rothman (1954, p 91) is of the opinion that the phenomenon of antidromic vasodilatation may be of great significance in the life-processes of the skin under physiological, as well as pathological, conditions He takes this view because, first, of its activation in response to local injury and, secondly because it may be a component in psychosomatic influences in inflammatory skin diseases. He seems to be of the opinion that emotional influences may act through central antidromic impulses with consequent vasodilatation. The vasodilatation, of course, then leads to an increase in the intensity of inflammatory processes, from whatever cause, and may also lead to a lowering of skin sensory thresholds. This latter effect has not been accepted by all investigators as a simple consequence of the vasodilatation, and other factors are regarded as essential to its occurrence particularly with reference to pain thresholds. The studies of Schumacher (1943) and Wertz (1942) may be cited as contradictory findings on this point. Rothman (1954, p 136) claimed that there is strong empirical evidence to support the view that itch thresholds are lowered by increased blood flow to the skin. He cites the observation that the diurnal peak temperature coincides with a greater tendency to itch. This peak occurs in the late afternoon. In contrast to this pattern, however it has been our experience in observing an extensive series of cases of pruritus vulvae et ani that the peak periods for itching occurred after retiring to bed at night and in the forenoon. Negative evidence which may be taken to provide some support for the view that skin vasodilatation lowers the threshold for itch is the finding that itching cannot be elicited in an area of skin where vasoconstriction has been induced by a local injection of epinephrine—Brack (1935) Melton & Shelley (1950). It is believed that the local anemia prevents impulses from arising in pain nerve endings. There is considerable evidence to support the view that itch and cutaneous pain sensations are mediated by the same nervous pathways. Rothman's (1943) admirable paper On the nature of itching summarizes the evidence. This association between pain and itch forms the basis of a very interesting paper by

Kepecs & Robin (1955) in which they point out the strong erotic component in itch and tickle sensations. They describe experiments which seem to provide some explanation of the role of masochistic drives in pruritic skin affections. I shall refer to their work again later

I have not referred to the physiology of vasoconstriction as this activity is subserved by the normal sympathetic fibres and has obvious relationships with the psychology of the emotions. One point may be made here, however in this connection. It is believed that the facilitation of vasoconstrictor mechanisms by adrenocortical hormones may account in part at least for their therapeutic effects in inflammatory skin reactions and may also explain their effect on pruritic conditions. Adrenocortical hormones could provide an essential link in the psychosomatic skin affections.

I have given this brief account of some of the physiological mechanisms involved in the vasomotor skin reactions because it may help in making a critical assessment of some of the research methods which have been used for studying the role of psychological factors in skin diseases. In a later paper Dr Ackner will be telling you of some of his own interesting researches on the effects of anxiety on cutaneous vasomotor functions and I shall not anticipate him here. I must, however refer to the work of Mittelman & Wolff (1943) These investigators made a careful study of the vasomotor activities of the skin in patients during psychoanalytic interviews. In each case preliminary control observations were made before interview In general they found that cutaneous vasoconstriction was associated with mounting tension and anxiety and vasodilatation with a lowering of tension after the discharge of emotion in the interview Some objection might be to their use of the radiometer for arriving at an estimate of blood flow but their findings are confirmed in an ingenious experiment by Kepecs, Robin & Brunner (1951) who observed the rate of exudation of fluid in an induced blister from which they had removed the top

I have chosen these two papers as illustrative of a class of experiments which are very numerous. Briefly they involve the observation of a variety of physiological changes which accompany changes of emotional state in patients or experimental subjects. Correlations of this kind are of great value. They do not, however take us very far towards an explanation of the mechanisms, psychological or physiological, by which these changes are brought about. There has been little attempt to use the approaches which have been devised to study the more complex processes involved in the establishment of behaviour patterns. Dr Russell Davis has referred to some of these psychological processes and I cannot go into them here in any detail. For instance, the vasoconstriction which is said to follow the fall in the level of anxiety raises the old question of the hen and the egg

Undoubtedly the tonic effect of cutaneous vasoconstriction may be said to increase the sense of well being but the mechanisms by which vasoconstriction takes place raises many further questions. We should like to know why in some cases a reduction of anxiety is only achieved by some patients when they can produce a widespread urticaria or when they have the localized vasodilatation of rosacea. Perhaps here the role of the skin in the expression of emotion is involved. This raises the further question as to how much previous experience or learning contributes to these reaction patterns.

We have, then, two apparently conflicting findings—a vasoconstriction which accompanies a fall in anxiety or which follows it and a reduction of tension or anxiety which follows the onset of symptoms in a disease process. This latter mechanism is one which seems fundamental to any psychological explanation of disease. Various theories have been proposed to explain it. Masserman (1955 pp 431–44), a leading experimenter and theorist in psychopathology elaborates four biodynamic principles which he parallels with the theories of psychoanalysis. His theories are expressed in the form of biological principles which would seem applicable to animals and to man. Principle IV of his scheme considers problems of conflict and anxiety. His summary of the biodynamic principle operating is as follows

When two or more urgent motivations are in sufficiently serious conflict so that the adaptive patterns attendant to each are mutually exclusive to the point of a paralysing impasse, then the organism experiences mounting tension and apprehension reaching various levels of anxiety while its somatic and muscular behaviour becomes either ambivalent, poorly adaptive and ineffectively substitutive (i.e. neurotic) or progressively more disorganized, regressive and bizarrely symbolic (i.e. psychotic) (p. 440).

This latter point about the bizarrely symbolic nature of some kinds of symptom formation, which he likens to psychotic reaction patterns, may help to explain some of the clinical problems one faces in the psychotherapy of the psychosomatic skin affections. Not infrequently a psychotic episode is associated with removal of the symptoms. However his formulation is still too vague to allow of adequate explanation of the mechanisms by which, say, conflicted motivations issue in the episodes of vasodilatation producing dermatological symptoms and lesions. Nor does it help to explain the chronicity of some of these conditions, nor their distribution. Here some conditioning or learning process might be invoked. The theoretical formulations of the mechanisms of learning in modern psychological theory could, perhaps, be applied with profit in the design of experiments on these problems. But we would like to know more about the nerve systems which subserve for example, antidromic vasodilatation. We know very little about

the central connections of this system. We are not clear as to whether the sensory fibres also carry the antidromic impulses or whether as Lewis (1942) believed, there are antidromic as well as sensory fibres. Perhaps Lewis's (1942) theory of a nocifensor nervous system could profitably be considered by theorists in the field of psychosomatic dermatology If we knew more about these processes we would be able to devise better models for the investigation of the fundamental problem—the translation of psychological conflicts into skin processes.

The question of the symbolism of symptoms depends on the personality structure of the individual case. This individual personality structure, of course, depends on memory and the previous experience and learning of the patient on the basis of his inherited capacities. The whole somatic system including the skin is no less involved in this learning process than the central nervous system. We do not know for instance, how much peripheral learning or conditioning is involved in the perpetuation of symptoms nor whether central processes play the major role in the chronicity of skin lesions of the psychosomatic type. It has recently been suggested that some sort of memory process in the spinal cord might explain cases of asymmetrical hyperhidrosis of clinical significance. A case which we investigated here recently might fit such an explanatory theory

The problem of personality is a very complex one and raises questions which are of fundamental importance in the planning of therapy for the psychosomatic skin disorders. Graham & Wolf (1950) studied a series of thirty cases of patients with urticaria. They found that their urticarial attacks were related to a particular kind of emotional disturbance and not to exposure to allergens. Almost all of their patients felt bitterly resentful of a situation which they could do nothing to resolve nor could they adequately express hostility This finding it will be noted, tells us about the current life situation of the patients which was regarded as the precipitating factor in the onset of the urticarial attacks. The patients were often quite aware of the feelings of resentment and of their origins. The investigators used the radiometer during special interviews to estimate the vasomotor activity of the skin. They also measured the threshold of reactive hyperaemia. Capillary tone was invariably lowered when feelings of resentment were aroused in the interviews. With hopelessness there was arteriolar constriction but a loss of capillary tone. With mounting anxiety there was both arteriolar and capillary constriction. They also attempted to elucidate some of the mechanisms involved in the production of the lesions and introduced histamine and pilocarpine into the skin of twenty four of their patients by electrophoresis. They also found that in these patients a feigned blow produced a dilatation of arterioles and a lowering of capillary tone just as

flushing of the skin is produced by a real blow. This latter finding at once raises questions of some kind of central learning mechanism. These investigators do not go into the question of the personality structure of their patients in any detail and the great outstanding question is why the skin of these particular patients should react in this highly specific way. Other studies of patients adopting a more historical approach to personality problems are those of Stokes, Kulchar & Pillsbury (1935), of Saul & Bernstein (1941), of Kaywin (1947) and the more recent one of Wittkower (1953) whose findings only partially confirm those of Graham & Wolf (1950). But personality studies of this kind do not help us a great deal in elucidating the mechanisms involved in the production of symptoms. Assessments of the personalities of patients using the projective techniques do not help with this problem either, although they may make classification of patients a little more justifiable. Good examples of this kind of study are those of Plesch (1951) who used the Rorschach test with fifty patients suffering from rosacea or morbid blushing, of Seitz & Gosman (1952) who used the Rosenzweig Picture-frustration test on pruritic patients. Studies of this kind are legion for all kinds of psychosomatic conditions. They render more objective, it is claimed, assessment of personality, but it should be pointed out that interpretation of the data elicited by these tests is dependent on the qualifications and experience of the interpreter and perhaps also on his own personality. They provide for the most part classificatory systems of personality traits and give us no direct evidence regarding the development of these traits nor of how they have come to be associated with skin lesions.

Studies which are centred on the association of specific personality types with specific kinds of skin lesion serve at best to emphasize the gaps which exist in our knowledge of these conditions. Only by linking theories of personality which can be verified experimentally, probably in a context of something akin to learning theory, with an accurate knowledge of the neurophysiology of skin processes will it be possible to make our descriptions of the psychosomatic skin affections continuous ones. Perhaps nowhere more than in this field is the historical approach to the individual case out of harmony with the kinds of generalizations which can be made from controlled experimental studies either in the laboratory or at the bedside. In this way, too, it should be possible to render the processes of psychotherapy more rational and communicable and less dependent on the individual experience and personality of the therapist. My own feeling is that these experiments should follow the important leads which have been offered us by such workers as Maier (1949), Masserman (1943, 1955), Mowrer (1950) and Liddell (1956). These experimenters, working mainly

with animals, have suggested an experimental methodology to us in the context of which studies of a great number of somatic manifestations and concomitants of behavioural disorders could be studied. Much work remains to be done here. There seems little reason to believe that their methods could not, with suitable modifications, be applied to the study of human subjects in the laboratory and I feel that this could be done without transgressing the ethically permissible limits. The work of Davis (1946 1948) on the disorganization of motor skills in human subjects in the Cambridge Cockpit revealed many examples of somatic reactions, such as sweating and flushing, which accompanied the conflicts aroused. These findings were not followed up at the time but they point the way to another useful approach.

These experimental methods, of course, do not primarily throw any direct light on the genesis of the personality structures of adult subjects, but they suggest hypotheses which could be tested in studies of the development of children. Genetic studies of this kind would also tell us a great deal about the establishment of links between central and peripheral neural mechanisms controlling cutaneous functions. The work of Seitz (1950) on psychocutaneous conditioning in the early weeks of life might be taken as an instance of how problems of this kind might be pursued. All experiments of this kind should take into account the available evidence about the underlying structural arrangements. For example, the cortical and hypothalamic connections of the autonomic system allow of the influence of central learning processes on the most peripheral functions of the skin circulation. This would have, in some way to be co-ordinated with those functions subserved by the widely ramified peripheral axon network which have some degree of independence of central control and may have learning or memory processes of a more elementary kind.

I have mentioned earlier the influence of variations in cutaneous blood flow on skin sensory thresholds. The physiology of skin sensory processes has not yet been at all fully worked out and the psychology of these processes is even less well developed. Equally important are the complex perceptual processes involving interpretation and meaning which must play a major role in the *formation and perpetuation* of pruritic lesions. This whole area is still largely virgin territory If our knowledge of these processes was even nearly as far advanced as that of visual perception we might be a great deal further on in understanding pruritus. Here the so-called new look in the psychology of perception which takes account of the influence of personality variables, has much to offer The symposium edited by Blake & Ramsey (1951) on *Perception An Approach to Personality* suggests many ideas for experimental study of skin perceptual processes by analogy with

the visual processes with which the work is largely concerned. *In short, in the words of Sir Frederic Bartlett (1932, p 33) temperament, interests and attitudes often direct the course and determine the content of perceiving*

The work of Kepecs & Robin (1955) to which I have already referred provides us with an admirable essay into this problem but it raises two questions which, *in more general form, could* be regarded as the central theme of this paper What are the psychophysiological processes which place the pain pleasure system of the skin at the service of erogenous masochism? What is the bridge between physiology and clinical experience? How complex even the peripheral mechanisms are can be judged from the *findings of Skouby (1952)* on the selective actions of acetylcholine and histamine on the peripheral receptors for itch and pain. There is also the itching of central origin.

I have referred to a mere fraction of the numerous studies of various skin disorders which are believed to have psychosomatic components. My approach has been mainly directed towards suggesting ways in which these various conditions might in general be regarded. I have not attempted any classificatory system. This may well be a useful exercise from time to time but can also be misleading One of the better classificatory systems is that of Stokes (1940). He describes nine possible mechanisms in the production of what he calls psychoneurogenous reactions of the skin. Classifications of this kind often derive from or have affinities with typological classifications of personalities. These in turn are usually based on a theory of inheritance of personality traits. I have made no mention so far of the role of heredity in the psychosomatic skin disorders. This is one approach to the question of the specificity of skin reaction patterns. Becker (1932) was an early theorist in this field but his theory of neurocirculatory instability based on a congenital or inherited protoplasmic hyperactivity throughout all of the body cells is difficult to accept. His attribution of general restlessness and hyperactivity to all patients with psychosomatic skin affections is not borne out by *clinical experience* and his theory of exhaustion as an explanation of the mechanisms is a decidedly unfashionable one in psychopathology today Wittkower & Edgell (1951) report a high incidence (up to 28 per cent) of hereditary predisposition to skin disease, coupled with respiratory affections in cases of what they call eczema *or more properly neurodermatitis. This they found to be* particularly so when the dermatitis began in the early years of life. Although they rightly distinguish between predisposing causes and precipitating causes of a stressful kind, the general criticism of studies of this kind, that the early interpersonal relationships in the family may be more important, applies here also. *Only more research* on the development of personality and of

the psychophysiological processes will help here. It has been suggested that the ectodermal embryological link between the skin and the cerebral cortex may account for the association of psychological abnormalities with skin disorders. Perhaps it would not be altogether without serious intent if I suggested an evolutionary link through the respiratory function of the frog's skin in the association of asthma and the skin lesion in atopic dermatitis! But, of course, we know that the epithelial lining of the respiratory tract is entodermal in origin and that its smooth muscle originates in the mesoderm.

Reference to embryological tissues inevitably reminds one of the great problem of malignant disease. It is not an infrequent clinical observation that emotional traumata seem to be associated with the onset of symptoms of malignant disease or with changes in the rate of its progress. The literature on psychosomatic aspects of neoplasia has been reviewed recently by Leshan & Worthington (1956) and by Petschke (1956) who found no fewer than seventy relevant publications. The idea that psychological influences could operate in this field may not seem so far fetched when we consider how far Selye's (1946) 'Theory of Stress and the General Adaptation Syndrome' has taken us into the innermost chemistry of living cells. I cannot comment on how acceptable his theories are to dermatologists but I have found Arnold's (1953) application of Selye's theories to diseases of the skin to be interesting for an outsider.

Before closing I feel I should refer to the most valuable and courageous book by Obermeyer (1955). *I feel he would not take it amiss if I admit to* believing that its most important chapters have still to be written.

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A CRITICAL ASSESSMENT OF THE PSYCHOSOMATIC CONCEPT APPLIED TO DERMATOLOGY

By F. A. WHITLOCK

At some future date a medical historian might relate with mild astonishment the interest taken during the past twenty to thirty years in the subject known as psychosomatic medicine. The interest is demonstrated by a number of well-established journals devoted to the examination of psychosomatic problems. A small library of books has been published on the same theme. If, as Alexander & French (1948) assert, all healthy and sick human functions are psychosomatic, it is difficult to see what justification there is in creating a special branch of medicine to deal with the matter. The statement is a commonplace which few people today would question but if the term psychosomatic is to be applied in such a wide and wholesale way it ceases to have any useful meaning beyond saying that all human functions involve mental and physical activities. Quite plainly there must be some limitation to the term in order that the problems raised by the consideration of body mind interaction can be fruitfully discussed.

The very term psychosomatic suggests a duality in the minds of the persons who use it. On the one hand there is organic medicine which is best understood in terms of biochemistry and physics. On the other hand are mental phenomena dealt with by psychologists and psychiatrists. Somewhere in between lies psychosomatic medicine bridging the hypothetical gap where these two fields of activity touch and interact. For my part I find it difficult to believe that such a gap exists and I doubt if the physicians of a generation or two back had much difficulty in dealing with these two aspects of human functioning as a single entity. There is nothing new in the knowledge that emotional disturbances are followed in some instances by physical complaints. What is new is the disbelief of some physicians in well-attested phenomena and, by contrast, the sweeping claims of other physicians who claim emotional disturbances as the prime aetiological factor in the genesis of all disease.

Two factors may have contributed to this state of affairs. The remarkable developments in physiology and bacteriology in the latter part of the nineteenth century may have led many to conclude that further knowledge on the same lines would lead to a total understanding and elimination of disease. The materialistic philosophy of the age was conducive to such

conclusions and the concept of the body *as a machine eliminated the need* for psychological considerations. However *the same period* which saw the development of modern organic medicine with its supporting sciences also saw the rise of psychoanalysis. *The reception of Freud's theories* at the hands of his medical colleagues is well known. It is difficult *to avoid the* conclusion that such disparagement has been responsible to a considerable degree for the divorce of psychoanalytic theory from the basic neurophysiological knowledge which surely must *be the foundation for any theory* dealing with mental phenomena. The results, so far as psychoanalysis is concerned, are shown by the flowering of *many strange and improbable* ideas untested by scientific examination. And organic medicine, turning its back on the mental life of its patients, lost sight of the human being as a whole while it concentrated its attentions on his separate limbs and organs.

It would appear therefore, that against the background of this split between psychology and organic medicine, coupled with a tacit belief in the duality of mind and body psychosomatic medicine came into existence. It was the psychoanalyst who made the running to develop basic psychosomatic concepts. Even to this day the greater part of psychosomatic theory research and practice are informed by psychoanalytic ideas without the investigator necessarily being a psychoanalyst himself I have no wish to attack psychoanalysis as such. But I do feel that much of the confused and confusing state of psychosomatic medicine today is due to the uncritical application of psychoanalytic concepts to the study of diseases thought to come into the field of psychosomatic medicine. Practically all basic ideas concerning the psychosomatic process derive from psychoanalytic observations and use its terminology In *some instances, such as* the use of the term physiological regression there is a direct and untested application of a psychoanalytic concept as explanation *of certain bodily dysfunctions.* It is my intention to *examine some* of these theories in the *light of more recent* observations. *If possible I hope* to use *clinical facts and* neurophysiological discoveries *to try to explain* certain *dermatological disorders, particularly in those cases where emotional disturbances* are thought to have *a direct aetiological significance.*

Lewis (1954) discussing the problem of *causality* and psychosomatic disease, *writes* As so *many of the writers* on psychosomatic subjects have been psychoanalysts this is a considerable problem (i.e. the causal relationship) they try to translate libidinal flux into physiological flux and imply a causal nexus With this comment I would wholeheartedly agree The early concepts of Freud and his followers held that repressed sexual and aggressive impulses would *in* certain circumstances become discharged through the autonomic nervous system giving rise to a variety of *physical*

dysfunctions. Inherent to this idea was the belief that the dysfunction itself in some way symbolized the underlying conflict. A discussion of this concept, therefore, may well be valuable.

The use of symbolism appears to be inherent in psychoanalytic doctrine. It is scarcely surprising, therefore, that it has been widely applied in the interpretation of psychosomatic disease with the consequent development of an 'organ language' in which each afflicted bodily system speaks silently but eloquently of the underlying emotional disturbances. Because the skin is visible for all to see this concept has been widely used with many references to folk lore, literature and the arts. For my part I become suspicious when a paper purporting to demonstrate the meaning of symptoms resorts to the Bible and Shakespeare to discover quotations to support its conclusions. That people 'blush with shame' or 'itch with fury' are among the clichés of our language. It seems quite unjustifiable to conclude from this that the physiological manifestations of erythema and pruritus are necessarily associated with these commonplace emotions. Kepecs and his colleagues (1951*a*) considered that the increased exudation of the skin in certain forms of eczema symbolized repressed weeping. In a later paper (1951*b*), however this finding was related more to the general reactivity of the skin. That there is little physiological relationship between tears and the serous exudate of an inflamed skin would scarcely be questioned today. What is astonishing is the facile nature of the comparison and the readiness with which it was accepted. Many analytic papers could be quoted in which similar inferences concerning the symbolic significance of a lesion are made. Kubie (1953, 1954), on the other hand, is unable to accept the specificity of a symbolic process and points out that most symbols are, in any case, over-determined. Macalpine (1954) likewise rejects the concept of symbolic meaning, finding that many psychosomatic symptoms are devoid of any obvious meaning whatsoever. She points out that a patient suffering from a psychosomatic disorder is neither psychotic nor psychoneurotic and differentiates between such symptoms and those of conversion hysteria. That such interpretations in psychotherapy have a certain usefulness is not to be denied. The danger lies in believing that what is pragmatically useful is also scientifically true.

Relevant to the subject of symbolic meaning is the concept developed by Alexander and his colleagues that each psychosomatic illness is related to a specific underlying conflict. Unfortunately few of Alexander's observations were made on skin patients, but it is difficult to believe that the fears and anxieties which trouble those suffering from hypertension are alien to those afflicted with eczema and urticaria. Indeed, when one considers the limited range of emotional experience one would scarcely expect the multi-

plicity of psychosomatic symptoms to be related in each case to a specific conflict situation. Love, hate, rage, resentment, envy, aggression and fear cover most of the possible emotional responses. Over-simplifications of this nature take no account of facts such as the variability of psychosomatic disorders in differing cultures as described by Linton (1956) or the variations within the culture as discussed by Morris & Titmuss (1944) and by Rennie & Srole (1956).

Complementary to the psychoanalytic approach is the method of somatotyping and personality profile studies developed by Dunbar (1943). It is doubtful indeed whether these studies are accepted today but as they have been widely applied in dermatology by Wittkower & Russell (1953), their study is relevant to this discussion. Briefly, it is maintained that specific disorders are related to fairly well-delineated personality types. The concept is founded upon the consideration of the life-history of the individual and upon psychological testing. Unfortunately, when the matter is put to the test it is difficult to discern any marked difference between the personalities and the peculiar life-situations which trouble a whole number of dermatological patients. Thus we read that the basic character of sufferers from eczema is that of the insecure, clinging child always in need of reassurance and affection. However, we also read that two-thirds of a group of patients suffering from urticaria stated that they missed parental and, especially, maternal affection as children. Discussing the childhood of sufferers from seborrhoeic eczema Wittkower writes, 'Whether spoiled or illtreated they always longed for love and still more love, and if they did not get it they felt frustrated and resentful.'

Quite plainly there is nothing specific about these situations and it is unjustifiable to draw any aetiological deductions therefrom. My choice of examples might be regarded as unfair but they could be multiplied indefinitely and there seems no point in adding to them. Both Mackenna (1944) and Hodgson (1945) have written correlating personality types with specific skin disorders or groups of disorders. Cormia (1947), however, is less certain of any real correlation between personality types and psychogenic dermatoses, believing that fundamental factors producing conflicts are of greater importance. For my part I am not able to discover any one personality type which can be associated with a given dermatosis. There is, however, a tendency in psychosomatic research to discover the factors for which one is seeking and it may well be that my scepticism about this type of approach colours my investigations, leading me to find an absence of significant findings where others have discovered them in profusion. As Mackenna & Macalpine in a later paper (1957) modified Mackenna's original views concerning the specificity of personality types for certain

dermatoses, regarding them as being associative without being necessarily of aetiological significance, I feel that one should keep an open mind to all psychosomatic theories however plausible. They are easy to propound but exceedingly difficult to prove.

A more recent theory developed by MacLeod, Wittkower & Margolin (1954) and Grinker (1953) concerns the concept of physiological regression. This appears to be a direct application of the concept of psychological regression to physiological dysfunction to explain the behaviour of the disordered organ or system. It is suggested that the infantile homeostatic mechanisms are of a relatively undifferentiated kind and that the organ affected by a psychosomatic disorder has regressed to this earlier and immature form of reaction.

The whole concept has been vigorously criticized by Mendelson and his colleagues (1956) who regard the concept as an uncritical application of a psychoanalytic doctrine to the field of physiology. Relevant to this topic is a paper by Grossman & Greenberg (1957) who studied the autonomic activity of new born infants and were unable to discover any deficiency of homeostatic regulations. Whatever value the theory may have for say gastroenterology it is difficult to find any application in the field of dermatology. I certainly do not feel that the infant's skin reacts differently from that of the adult except in so far as the lack of previous exposure to trauma and infection modifies allergic responses. If as I believe, itching is an essential feature of psychosomatic skin disease, it is difficult to see in what way such a symptom can be regarded as a regressive one. I would conclude, therefore, that the concept is unsupported by clinical observation and devoid of heuristic value.

These comments may well give the impression that I am hostile to the psychosomatic concept as well as to psychoanalysis. This is far from being the truth. Psychiatry and general medicine have both been enriched by the discoveries and tenets of psychoanalysis. It would be flying in the face of clinical experience to deny that much disease is closely connected to preceding emotional disturbance. I do feel, however that the attempts to explain these dysfunctions by psychoanalytic interpretations have not been successful and, in many instances, are a bar to progress. So often one gains the impression that once such an interpretation has been made the whole problem has become explained. The neurophysiological linkages between the emotional event and the bodily dysfunction are not considered and all that is inconvenient to the theory is omitted. It appears to me so obvious that *any* explanation of *any* disease must make use of multiple concepts that it is difficult to understand how theories embodying one approach to the exclusion of all others ever came to be considered. A psychoanalytic explanation quite clearly omits all reference to constitution and the

interaction with the environment except on a most limited scale. Cultural and social factors have to be ignored and physiological facts appear to find little place in psychoanalytic theory. If we are asked to believe that a given skin disorder symbolizes an underlying emotional disturbance we are entitled to ask how this comes about. That we are ignorant is only too true. But this should not stop us from asking the question, and it is my opinion that the failure to ask such a question is one of the prime reasons why psychoanalysts has not succeeded in interpreting psychosomatic disease.

It would be pretentious to claim that I am able to supplant these theories by anything more comprehensive and definite. Nevertheless I would like to consider certain concepts which might have some bearing on the problems of psychosomatic skin disease. These comprise the theory of central itching, the concept of feed-back and the belief as already stated, that any attempt to account for psychosomatic disease must be multifactorial and include physiological and biochemical facts upon which any unifying theory can be based. This last demand is an extensive one which, in the present state of our knowledge, cannot be met.

I have previously (Whitlock, 1957) discussed the physiology of central itching and must emphasize that the hypothesis put forward was a tentative one still unproved by experiment. Nevertheless, it is a theory which provides some explanation for a variety of dermatological phenomena. I am fairly convinced that any emotionally precipitated dermatosis is pruritic. If this is so one might expect that central itching is of considerable importance in the production and perpetuation of the dermatosis even when the site of the lesion is determined in the first place by local trauma or infection. Without invoking some central mechanism influenced by emotional disturbances it is difficult to see how some dermatoses persist for such lengths of time impervious to applications which would have healed a lesion caused solely by local noxae. If one is to accept that emotional disturbance is responsible for the continuation of the illness one must assume some central locus for its effects to manifest themselves. Nobody for instance, would assume that hypnotically produced skin lesions or sensations are caused by the effect of suggestion on the peripheral tissues. Quite plainly the action is a central one and the lesions themselves are secondary to central change.

Although I emphasize the importance of itching I do not wish to dismiss as irrelevant other aspects of skin function. However I think one should separate clearly the vasomotor sweat and other autonomic activity which are the normal concomitants of anxiety from itching and erythema which are not usually associated with this common emotional experience. It is natural to sweat, to become pale or blush in certain circumstances. I feel

that these physiological accompaniments of anxiety are qualitatively dif ferent from skin disorders which might be supposed to be related to some non-specific emotional disturbance.

It might be added here that, because of my conviction that dermatoses of emotional origin itch, I would eliminate from the field such conditions as acne rosacea, alopecia areata and vitiligo. The inclusion of acne rosacea appears to rest largely on a paper by Klaber & Wittkower (1939) which was a personality profile study without adequate control groups. I have not been able to confirm their findings in any patients with this disorder whom I have examined. I would agree with Macalpine (1958) who could find no evidence to ascribe alopecia areata to emotional disturbances. The close association between alopecia and vitiligo make it probable that this disturbance of pigmentation is not due to psychic factors. It could be argued that Raynaud's disease, which does not usually itch, is often precipitated by emotional crises. This condition, though, appears to be primarily a vasomotor disturbance with only secondary involvement of the skin in cases of some severity

Relevant to this concept of central itching is that of feed-back which is merely a convenient term for describing the self-regulatory activities of the human body Most of the theories discussed in the earlier part of this paper appear to imply a direct causal linkage between one or more factors without analysing the manner in which each of the components in the causal chain interact and modify each other That interaction occurs is obvious, both on the physiological and psychological levels. Failure to make allowance for such interaction explains much of the controversy centred upon whether emotional disturbance produces the dermatosis or whether the tension and distress is the reaction to any unpleasant disease. The work of Granit (1955) on centrifugal action-potentials modifying the nature of incoming neuronal messages points to the possibility of the influence of incoming sensory impulses by the general emotional state of the individual. It must be admitted that, so far there is no direct evidence that these centrifugal impulses ever enhance sensation or create a summation of impulses. This remains an unproved possibility in the field of somatic sensation, but if such activity could be demonstrated it might have a very real bearing on some of the problems of pruritus and other skin sensations.

From my remarks hitherto it should be plain that I would not expect a point-to-point correlation between specific emotional event and the production of a dermatosis as such expectations are not supported by clinical experience. Possibly one of the greatest impediments to psychosomatic research has been the attempt to explain the development of an illness by reference to one exclusive aetiological factor The use of a personality

profile or a somatotype quite plainly is inadequate to account for the genesis of an illness, leaving out, as it does, the social and cultural circumstances, the previous life experience, the present emotional situation and the previous exposure to psychological and physical traumae. All these factors and others besides require consideration before assessing the responsibility of any one as being the main aetiological agent. Reference to two diseases, chronic urticaria and lichen simplex, may make some of these concepts clearer

In a recent study (1957) Linford Rees stresses the multiplicity of factors in the aetiology of chronic urticaria. He was unable to isolate any specific emotional factor or personality type developing this disorder. From my own limited experience I would agree with these findings. What also appears plain when studying chronic urticaria is the manner in which modification of one factor appears to modify others. So frequently one finds chronic urticaria precipitated by exposure to a definite allergen. The allergen is withdrawn but the urticaria persists. Further examination reveals that prior to the breakdown there existed a state of chronic emotional tension which is now persisting. Treatment of the psychological condition by psychotherapy may lead to improvement or cessation of the urticarial attacks. It is then found that the allergen no longer is able to produce the former symptoms of itching and whealing. This observation appears to show that the persistence of the urticaria could be attributed to the underlying emotional tension but that the illness is precipitated by a physical cause. Once this has happened the emotional disturbance appears in some manner to alter the reactivity of the skin, maintaining its sensitivity to the allergen until the emotional factor is eliminated. It is not always easy to discover whether the whealing and itching in this disorder is the cause or the response to scratching. But if the concept of central itching is a valid one I would not be unduly surprised if the initial symptom is itching and that the greater sensitivity of the skin to trauma produces the subsequent lesions. We have all seen pronounced urticarial whealing without any complaint of pruritus. We have also seen the converse phenomenon. I feel that in many cases the itching is central in origin in response to the emotional disturbance which, likewise, must be centrally located.

Lichen simplex is another illness where emotional tension appears to be one of many factors of aetiological significance. I see no real reason to separate this disorder from many cases of anogenital pruritus where the lesion often appears as a patch of lichen simplex differing in no way from the appearance of the familiar suboccipital dermatosis. Only the situation varies and I hope to show that the choice of site is largely fortuitous without necessarily signifying some deep psychological meaning

The well-demarcated lesion of lichen simplex often originates after some trivial trauma such as a scratch, an insect bite or a minor infection. The comparison between this and the allergen precipitating urticaria is fairly obvious. Once the initial lesion has, as it were, determined the site, itching and scratching will do the rest. Again one is faced by a circular effect best explained by the feed-back principle. Emotional tension leads to central itching which is projected to an area already made vulnerable by some degree of peripheral itching consequent to the primary injury. That the situation in the anogenital area is a common one is due, I think, to the greater vulnerability of this area to minor infections and traumae as well as to the heightened emotional significance of the parts involved. It may well be that many cases of anogenital pruritus are the result of underlying psychosexual tension, but I would not agree with Sharvon (1950) and Wittkower (1950) who maintain that in all these cases the sexual conflict is paramount. I would agree more with Seitz (1954) who affirms that hostile aggressive conflicts are of equal significance.

If any therapeutic conclusions are to be drawn from these observations I would suggest that they centre on the need for close co-operation between psychiatrist and dermatologist, both of whom should have a fair understanding of each other's problems. Not all cases of dermatoses with no known cause can be accounted for by invoking emotional disturbances. By no means will all of those cases where emotional factors appear to be of importance respond to psychiatric treatment alone. Any attempt to make a given dermatosis the exclusive domain of the psychiatrist or the dermatologist is unlikely to succeed. The causes of these illnesses are manifold and so, I believe, are the cures.

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THE RELATIONSHIP BETWEEN ANXIETY ALERTING AND CUTANEOUS VASOMOTOR ACTIVITY

By BRIAN ACKNER

PROBLEMS OF METHODOLOGY

The study of the relationship between emotional disturbance and circulatory changes involves difficulties which are common to all attempts to correlate mental states with bodily changes. It is difficult to characterize exactly the feelings experienced by individuals and subjective states cannot be quantified. The behavioural expression of emotion can be more easily described but it is affected by cultural factors and influenced by the immediate environmental situation. Disturbed mental states can be experimentally produced, but are difficult to reproduce and certainly do not bear accurate comparison from one individual to another. Subjects can be exposed to comparable stimuli or environmental factors, but it is the emotional significance of the latter to the individual which determines whether at any particular time they will be stressful or not. The external factors producing emotional stress can be described but an emotional stressor cannot be quantified.

To the many difficulties of psychological origin must be added those derived from the complexity of circulatory physiology. The circulation may be influenced by many factors which include variations in heart-rate and output, changes in arterial and venous pressure, variations in the capacity of the circulatory bed, respiratory irregularities, alterations in metabolism, the need for temperature regulation and changes in posture. If the relation between emotional disturbance and circulatory changes are to be studied accurately many of the above factors must be held constant and to attempt this adequately may severely restrict the scope of the investigation.

Restrictions may also be imposed by the need to avoid what Burton (1954) has described as the 'reactive error in physiology', namely the reaction of the organism to the stimulus of the application of the method of measurement. The avoidance of this error is particularly important in any investigation involving emotional factors and necessitates the use of apparatus which is simple and easy to apply, together with methods of recording which are as unobtrusive as possible.

FINGER PLETHYSMOGRAPHY

Fortunately many of these requirements are met by the technique of finger plethysmography The volume changes occurring in the finger-tip with each pulsation are recorded within a closed system which consists of a suitable glass cup fitting over the finger tip and connected to an electronic capacity manometer by means of plastic tubing Suitable calibration and amplification systems enable the size of the pulse and finger volume changes to be measured from the tracings of a continuous pen recording apparatus. As the blood supply to the finger does not include vessels to muscular tissues, cutaneous vasomotor reactions are recorded using this technique. In contrast to limb plethysmography where muscle vessels are also involved, blood pressure changes do not appear to be of much significance in evaluating changes observed in the finger plethysmograph (Burton, 1939 Abramson & Ferris, 1940 Neumann, 1943 Mathes, 1952). There is no evidence of dilator fibres in the digits and the innervation is entirely sympathetic in origin (Warren, Walter Romano & Stead, 1942 Arnott & Macfie, 1948 Bridges & Yahr 1955). After an adequate stimulus (for example noise, light, pain, a threat) the pulse and finger volume is decreased, with a latent period of 3–4 sec. whatever effective stimulus is employed. This response is absent in the sympathectomized limb. The pulse volume waves are related to the heart beat but their size is largely dependent on the degree of vascular dilatation. When examination is carried out under suitable temperature conditions, with the limb maintained at heart level and variations in venous pressure are avoided, there is then a very close correlation between the amplitude of the pulse volume waves and the blood flow of the finger (Burton, 1939 Melrose, Lynn, Rambow & Wherrell, 1954). Under these conditions variations in the pulse amplitude can be used as a direct index of changes in the vasomotor tone of the skin arterioles.

THE RELATIONSHIP BETWEEN ANXIETY AND CUTANEOUS VASOMOTOR ACTIVITY

Previous workers (Van der Meuve & Theron 1947 Theron, 1948 Van der Meuve, 1948) have shown that anxious subjects manifest a relative peripheral vasoconstriction as evidenced by their finger plethysmographic pulse volumes being smaller than in relaxed subjects. However the tendency was only a statistical one and the overlap was such that the absolute pulse volume could not be usefully used to differentiate between emotionally disturbed and relaxed individuals. This is to be expected having regard to

the many factors, other than emotional, which influence the final size of the peripheral finger pulse volume.

It occurred to the author that induced mental relaxation should reduce the vasoconstriction associated with emotion with resulting vasodilatation in emotionally disturbed subjects but little change in those who were already relaxed. Thus, rather than vasoconstriction the tendency to vasodilatation might be found, under suitable conditions, to differentiate between emotionally disturbed and relaxed subjects.

After examining a number of alternatives it was decided that sleep induced by oral barbiturates was the best means for producing a state of mental relaxation during which subjects could be suitably compared. Certain experimental conditions, particularly with regard to temperature, time of day diet, etc., needed to be fulfilled and the details of the experimental procedure have already been reported in full (Ackner 1956*a*, *b*). A group of patients was selected on the basis of subjective complaints, appearance and behaviour all of which left no doubt that the term severe anxiety as generally used, appropriately described an aspect of their emotional state. In other words, this was a markedly anxious group as judged by both the common internal and external criteria. This group was compared for experimental purposes with a normal control group and with another patient group, in both of which it was considered that the internal and external criteria of anxiety were absent.

The experimental findings revealed that the three groups differed significantly both in the size of the mean pulse volumes during the resting state and in the increase in the mean pulse volumes occurring during induced sleep

Pulse volume changes

	Mean initial pulse volume (per 5 c. finger tip) (cu. mm.)	Mean pulse-volume increase (per 5 c.c. finger tip) (cu. mm.)
Resting tests		
Controls	9·4	·0
Non-anxiety patients	5·8	·6
Anxiety patients	3·4	·0
Sleep tests		
Controls	0·	0·55
Non-anxiety patients	6·3	1·3
Anxiety patients	4·9	2·3

The marked pulse-volume increase during induced sleep occurring in the anxiety group completely distinguished all these subjects from those in the other two groups.

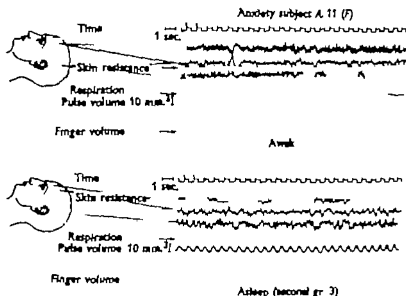


Fig. 1 Anxiety subject A. (F).

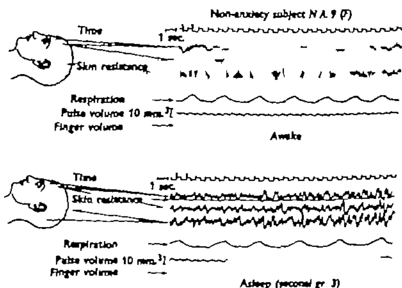


Fig. 2. Non-anxiety subject N.A. 9 (F).

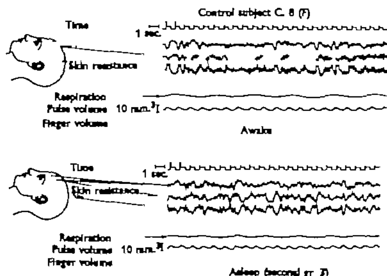


Fig. 3. Control subject C 8 (F).

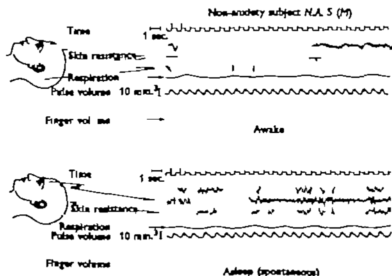


Fig. 4. Non-anxiety subject N.A. 5 (M).

Range of pulse volume increase during deep (per 5 c.c. finger-tip)

	(cu. mm.)
Controls	0–1·5
Non-anxiety patients	0–5·0
Anxiety patients	6–23·0

It should be emphasized that whilst the release of this emotional vasoconstrictor factor in the skin vessels of the limbs can be profitably used in the study of anxiety it cannot be so used for the purpose of measuring the degree of anxiety present. Whilst it is not difficult to reach common agreement as to the application of the term anxiety to a group of subjects suffering from marked and overt signs and symptoms, it is far from easy to achieve a satisfactory method of rating the degree of anxiety present. The fact is, of course, that anxiety is a rather vague term often loosely used in a generic way to cover a variety of subjective complaints and objective signs. Anxiety is not an entity but is a term which is applied to a state which can be qualified but not effectively quantified.

THE RELATIONSHIP BETWEEN ALERTING AND CUTANEOUS VASOMOTOR ACTIVITY

During the course of the study just described other interesting findings emerged. As the subject began to relax the pulse-volume waves tended to show periods of decrease in amplitude lasting a few seconds. With increasing relaxation and drowsiness these fluctuations often took on a more periodic character sometimes occurring two or three times a minute.

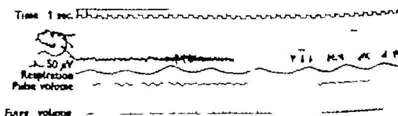


Fig. 5. Subject (Miss O.M.) drowsy (no drugs). The return of alpha rhythm is followed by diminution of the amplitude of the pulse volume waves on two occasions. On the first occasion there is change in depth of respiration while on the second occasion there no change.

These were most marked during the early stages of sleep and, as sleep deepened, tended to lose their periodicity becoming more episodic in character. Such phenomena had, however, been noted as long ago as the latter part of the last century by workers using brain and limb plethysmography in an attempt to study the dynamics of the cerebral circulation. These fluctuations were then thought to be due to Traube–Hering blood-

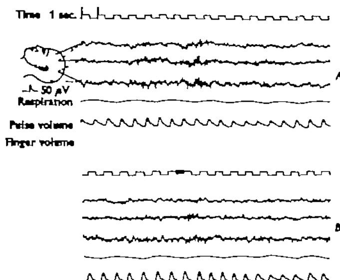


Fig. 6. Subject (Mrs C.) drowsy after seconal gr 3 (*A*) Spontaneous return of alpha rhythms followed by vasoconstriction. (*B*) Evoked return of alpha rhythms is followed by vasoconstriction with delay similar to the spontaneous response in (*A*) above. (Stimulus indicated by black area on time marker.)

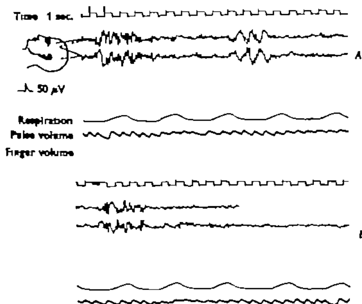


Fig. 7 Subject (Mrs S.) asleep after seconal gr 3 (*A*) Two spontaneous K-complexes are followed by vasoconstriction. (*B*) K-complex elicited by stimulus is followed by vasoconstriction with delay similar to the spontaneous response in (*A*) above. (Stimulus indicated by black area on time marker.)

pressure waves though later workers offered different explanations. Ingram (1936) and Burton (1939) considered the fluctuations to be serving temperature control. Abramson & Katzenstein (1941) regarded them as spontaneous peripheral changes, whilst Neuman, Lhamon & Cohen (1944) held that they were a reflexion of variations in sympathetic tone in relaxing subjects.

In a combined E.E.G. and plethysmographic study (Ackner & Pampiglione, 1957) it was possible to demonstrate that, during relaxation, drowsiness and early sleep many of the spontaneous plethysmographic fluctuations were preceded in a constant time relationship by E.E.G. changes commonly accepted as being associated with arousal (for example, return of alpha rhythms, appearance of K-complexes). Furthermore the morphology of the spontaneous E.E.G. changes preceding the spontaneous vasoconstrictions varied according to the level of alertness or sleep in just the same way as did the E.E.G. responses evoked by alerting stimuli (see Figs. 5–7).

It thus appears probable that many of the apparently spontaneous vasoconstrictions in the plethysmographic record, which have formerly been ascribed to a variety of causes, are really related to fluctuations in the levels of alertness or sleep.

DISCUSSION

Presumably vasoconstriction of the skin vessels on an emotional basis is part of a bodily alerting reaction to stress, probably serving the function of diverting blood to areas which are becoming mobilized for offensive or defensive action. Anxiety is a state in which a heightened alertness is sustained and thus is associated with a sustained peripheral vasoconstriction. Common to all the stimuli capable of evoking a vasoconstrictor response is the capacity of the stimulus to arouse the immediate attention of the individual. Once this arousal has occurred further stimuli prove decreasingly effective. But it is not necessarily the conscious attention of the individual which has to be aroused, for the reaction is present in the absence of consciousness. Peripheral vasoconstriction is an extremely sensitive response which will still occur at a depth of sleep at which other bodily reactions, such as skin resistance, respiration and heart rate, fail to respond by any change (Ackner & Pampiglione, 1955). Furthermore, even during sleep, stimuli will vary in their effectiveness according to their nature and significance to the individual and adaptation still occurs, although occurring much more slowly than during the waking state (Pampiglione & Ackner, 1958).

Time does not permit a full discussion of the relationship of the findings I have described to the so-called psychosomatic skin affections. It should be emphasized that the skin vessels of the face do not react similarly to those

in the limbs and that capillary and venular responses complicate the picture. Anxiety is known to be associated with an increased susceptibility to Raynaud's Syndrome, frost-bite and trench foot and the relationship to emotional vasoconstriction is here clear I suspect that in some cases of urticaria and angioneurotic oedema the disturbance occurs following a period of stress and is associated with a rebound vasodilatation caused by the release of emotional vasoconstriction during relaxation. The circulatory dynamics of many other disorders remain to be elucidated.

SUMMARY

Investigations are described in which combined E.E.G and finger plethysmographic recordings were taken from emotionally disturbed and normal control subjects. Anxious subjects manifest a relatively increased cutaneous vasoconstriction and significantly differ from normal control subjects in the degree of vasodilatation occurring during sleep induced by oral barbiturates. Stimuli having an alerting significance to the individual induce cutaneous vasoconstriction both in the waking and sleeping state. Spontaneous fluctuations in the level of alertness or sleep tend to be accompanied by changes in cutaneous vasomotor activity

The significance of these findings for some of the psychosomatic skin affections is briefly considered.

ACKNOWLEDGEMENTS

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Figs. 5 6 and 7 are reproduced by permission of the *Journal of Neurology Neurosurgery and Psychiatry*

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DISCUSSION

Chairman DR BRIAN RUSSELL (London)

RUSSELL. As a simple dermatologist I reason in this way Man is an aggressive and lustful being The poker faced individual may have skin symptoms and signs because he is masking his drives. The secondary gain apparently being derived by some patients from their dermatoses impresses me so much that I am inclined to divide patients into two categories those who wish to get well and those who (unconsciously) resist cure, sometimes aggressively putting the blame on the doctor sometimes with the classical *belle indifference* of the hysteric.

DR D RUSSELL DAVIS (Cambridge). Dr Russell has suggested that secondary gain may account for the prolongation or the relapse of skin disorders, *as the patients are leaving hospital in particular but the problem* remains How does this reward which is of a rather complicated psychological kind, produce the actual skin lesion?

RUSSELL. Are itching and scratching synonymous? We know that patients with *acne necrotica* and neurotic excoriations may scratch without any apparent itching and I for one believe there are many patients with Besnier's prurigo who do not necessarily have intense pruritus at the same time as they are scratching their lesions. In this connection we have experimental work on cats where injections have been made into the brain and violent scratching movements follow Does that necessarily mean that those cats are having the sensation of itching?

DR F A. WHITLOCK (Newcastle). Dr Russell has raised the question of whether the scratching one sees in response to intracisternal drugs in these animals—I refer of course to the experiments of Koenigstein (1948) and of Feldberg & Sherwood (1954)—is really a response to itching It is quite impossible to give a definite answer but from the description of the animals one certainly gets an impression that they are itching This appears to be a state of itching due to a central nervous infection and it should be possible to judge whether the animal s movements are due to itching or to some other sensation.

DR J H. CULLEN (Cambridge). Central components of the perceptual processes involved in itching are very important in the pruritic skin conditions. However clinical observation, which is often very misleading, suggests that in some cases the skin changes anticipate the changes in the perceptual and sensory processes while in others the skin changes are preceded by sensory disturbances. These observations are based largely on

descriptions by patients and are very difficult to assess critically They suggest that we need to do a lot more in the way of controlled experimentation to decide upon these matters.

RUSSELL. Surely a person under emotional conflict generates intense fatigue—we are all familiar with the relief we get when we decide upon a course of action and how our fatigue passes off It is only a subjective phenomenon but nevertheless can it have some organic cause from adrenal-medullary exhaustion due to prolonged emotional stimulus? I would like to ask the speakers if they would care to come in on any of those points.

RUSSELL DAVIS. *As I have said in previous discussions on fatigue, the term has now no precise meaning in psychology except a soldier's non-military duty!*

DR R. P WARIN (Bristol). I would like to comment on the case that Dr Whitlock mentioned of chronic urticaria which apparently was started with aspirin but later on aspirin did not have any effect. In a number of cases of chronic urticaria, from whatever cause, aspirin will produce exacerbations of the urticaria. It does not appear to be due to an allergy to aspirin but is a quantitative effect of salicylates. At a later stage when the tendency to urticaria is less the same dose of aspirin may not give an exacerbation.

I would also like to support him in his view that a lot of cases of chronic urticaria start with some acute episode, often a food or drug allergy and the condition then drags on for months and months, and behaves exactly like other cases of chronic urticaria without such an acute onset.

DR G C. WELLS (London). When this increase in blood flow to the hand occurs in an anxiety subject on going to sleep, how long is it sustained? Is it comparable with reactionary hyperaemia following cold?

DR B C. G ACKNER (London). I would like to emphasize that all subjects under normal rather cold temperature conditions will vasodilatate as they go to sleep and observation will confirm that they are flushed during sleep but I was referring to cases which were examined under higher temperature conditions and the dilatation was then manifested largely in the anxiety subjects. In this group as in the normal the vasodilatation begins usually just before the sleeping stage and reaches its maximum perhaps just after sleep has been reached, persisting for a while until a gradual vasoconstriction begins. If you leave the subject sleeping all the night this vasoconstriction gradually increases until the waking state occurs. Of course if you wake the person up suddenly there is then a massive vasoconstriction.

DR P A G MONRO (Cambridge). *May I add my commendations to* Dr Ackner on his most interesting results. I have made a number of

finger plethysmographic observations but this factor of variations in size of the pulse wave forces me to conclude that almost all my patients were half-asleep! There seem to be many other factors affecting these variations in the pulse wave. There is, of course, a large literature on this subject already but I have not previously heard of any observations combined with electro-encephalographic observations. I would like to inquire as to the ambient temperature at which these patients were examined, and for how long they had been maintained at this temperature. In this country there is a shortage of constant temperature controlled rooms, but in America where their facilities are rather more lavish, patients who are to undergo plethysmographic observations are allowed as long as 6 hr to adjust themselves to a constant temperature in the hope that they will produce more constant digital blood flows and pulse waves. I have found that with sympathectomized subjects, the variation in size of the pulse wave from minute to minute is less than it is on the opposite unsympathectomized normal side, but it is still apparent. I have also found that in patients with peripheral vascular disease the relationship between the size of the pulse wave and the digital blood flow per unit length of finger is very variable, and I cannot agree that in these cases it shows a constant relationship. There is a relationship of course, but if you plot these values against each other there is considerable scatter

My last point concerns the delay between the stimulus, such as an audible stimulus, and the appearance of the psycho-galvanic reflex. This may be detected by measuring the skin s electrical resistance or by measuring the vasoconstriction. Some authorities seem to have thought that the delay is due to the reaction time in the perception of the stimulus, but if you stimulate a sympathetic ganglion directly as I have done during lumbar sympathectomy you still get a delay of about 2 sec. from the start of the stimulus to the onset of vasoconstriction. Geohegan *et al.* (1942) have shown in the cat that if you stimulate the anterior nerve root you get the same order of delay in the appearance of the sudomotor response. It means that although there may be a reaction time in the appreciation of a stimulus, the greater part of this delay is in the production of the effect at the periphery

ACKNER. The temperature in these patients was always maintained above 22 C. During their resting runs they were allowed to rest for some hours. All patients and subjects who gave a history of sensitivity to cold or cold vasoconstriction, were excluded from these investigations. During the actual experiments patients were tested first thing in the morning while they were still warm soon after they had got out of bed and before they had been exposed to cold conditions or emotional influences. They were

left for at least an hour before the experiment was started and certainly longer if there appeared to be any tendency for vasodilatation to be occurring in relation to the temperature conditions of the experiment. I would agree that in sympathectomized subjects you can get vasoconstriction occurring, but previous work some years ago by Freeman, Smithwick & White (1934) showed that the delay following stimulus was much longer than in the non-sympathectomized limb and it was concluded that this type of vasoconstriction was due to the sympathectomized vessel being more sensitive to circulating adrenalin. I would agree that in peripheral vascular disease you get a different picture, but I think that that is because the vascular dynamics in such disorders have been complicated. I would also agree that the delay is a peripheral phenomenon and this is suggested by the fact that the reaction time, whether you are measuring a vasoconstrictor response or a psycho-galvanic response is longer in the toe than in the finger there being a longer nervous pathway for the impulse to travel.

DR G B MITCHELL HEGGS (London). I do not know whether Dr Ackner has been able to do any of his experiments in patients who have had hemiplegia. I have been impressed by two patients who have lost their pruritus after in one case, a stroke, and in another case a leucotomy

ACKNER. I have not done any such experiment myself they were done many years ago by Stürup Bolton Williams & Carmichael (1935), and the actual reflex pathway for the vasoconstriction was shown to be complete below the level of the thalamus. The cortex will be involved in emotional vasoconstriction, though not necessarily so because I think our responses to various phenomena do not occur solely at the level of the cortex.

DR C. H. WHITTLE (Cambridge) I agree. Dr Ackner s work is most interesting and stimulating, but I feel some misgivings about the sharp distinction it leads him to draw between the anxiety patient and the normal and I am even less happy about Dr Davis s separation of the neurotic from the normal person. We have fallen into the easy habit of using this word neurotic to detach the patient from us and protect us from too close a contact, probably because we ourselves are not too secure but whether this helps in our treatment of the patient s disorder is another question.

ACKNER. Dr Whittle suggests that there must be a catch in this because it is too neat a distinction and I must say I was myself worried for some time. But I would emphasize that the subjects I have studied were a group of extremely severe anxiety patients compared with others who were not anxious. There was no attempt to measure anxiety and I would have been very suspicious myself if I could have found a correlation between the degree of vasomotor change and the degree of anxiety because I do not agree that you can grade anxiety However I compared two different

groups, those in which there was an extreme amount of anxiety present and those in which it was absent. I do not think I ever used the word neurotic or suggested that I was dividing the population into those who were neurotic and rather nasty and those who were normal. In order to control the situation, I did study another group who were composed of recovered depressives, recovered hysterics and so on, who were not anxious at all. Therefore the distinction was not between people who were neurotic and people who were not, but between various subjects showing marked anxiety and those who showed none at all. In fact these clear correlations do not occur if, instead of putting the patients to sleep by giving them a sedative which they regard as a rather enjoyable rest period in the morning, you approach them with a syringe full of amytal, and put them to sleep that way The correlations then become rather mixed up because you are dealing with an induced stress situation, whereas I started off with a situation which was inducing relaxation.

Dr A. J E. Barlow (Huddersfield). I hope the speakers will forgive me for asking a rather simple question, but I got rather lost in the psychological terminology which seems infinitely more complicated to me than dermatological jargon. I gather there are such things as normal people, there are a group of people with psychosomatic manifestations, a group whom one describes as psychoneurotic, and a group whom one describes as psychotic. I wondered if one of the speakers would define where this begins and ends, and I assume that they are not genetically determined like staphylococcal phage types and therefore constant.

Russell Davis. Can one make a differentiation between normal, neurotic and psychotic? I suspect Dr Barlow is only teasing me and does not expect a full reply but I had better indicate my position. No, I do not think one can properly distinguish between individuals, but one can distinguish between adaptive, neurotic and psychotic mechanisms. Normal people have both neurotic and psychotic mechanisms. The people that we loosely call neurotic or psychotic have them in a tiresome or distressing way

Whittle. I feel that Dr Whitlock's summary his discussion, and his rather destructive criticism are salutary and that we ought to take it to heart I feel it is the beginning of the right line.

Russell Davis. Dr Whittle expressed a preference for Dr Whitlock's views with his destructive outlook. I have not made the impression I wished to, it seems. I listened to Dr Whitlock very carefully I did not feel there were any real differences between us, except in so far as I did express the opinion that those who suffer from psychosomatic disorders tend to be of neurotic personality I was at that time quoting the general view of authorities. I did not give this opinion as my own. I do think there

is some truth in the opinion that people who suffer from psychoneurotic disorders tend to have neurotic mechanisms to the fore. At any rate, from the research point of view the problem is to correlate neurotic mechanisms with somatic manifestations.

WHITTLE. I would like to ask Dr Russell Davis what is the relative importance of the environmental factors as against the genetic ones. Long ago the Jesuits maintained that any influence they wished to exert in a child's development would be established within the first five years of his life.

RUSSELL DAVIS. Inherited factors play a certain role. I am by trade a psychologist. So I am concerned with the factors in the psychological environment which, acting on a person of particular constitution, produce stereotyped patterns of reaction. For this reason, I have not paid any direct attention to inborn errors of metabolism. I have no doubt that they are of an importance—though they have yet to be defined. Amino-acid metabolism is likely to be a useful point of attack.

DR F RAY BETTLEY (London). Over the last ten years I have seen all the skin cases in a large mental hospital, with a changing population of about 2000 There has seemed to be a complete lack of special incidence in that population of any psychosomatic or supposedly psychosomatic dermatoses, and I wonder if any speaker would comment on this. Rosacea is a cutaneous disorder which I have often thought was sometimes rather convincingly psychosomatic, but of the two cases of rosacea I have seen in this mental hospital in ten years, one was an advanced elderly arteriosclerotic dement, with apparently no emotional existence at all and the other was a very euphoric G.P I —neither in the least like the guilt ridden obsessional one is sometimes led to expect.

DR P BORRIE (London). I quite agree with Dr Bettley that mental hospital patients have an extremely low skin morbidity with the single exception that alopecia areata is commoner in mongols than in the normal population.

MITCHELL HEGGS. Unfortunately my experience of lunatics is of those who are in a criminal lunatic asylum, but I join with the others in saying how amazed I have been that there are such a small number of patients suffering from itching disorders.

RUSSELL. Patients in mental hospitals are now well fed and well looked after and are freed from outside problems. Often our patients do well in hospital but their skin phenomena recur when they have to face up to reality again.

DR D S WILKINSON (Amersham). The very interesting experience of Dr Bettley and Dr Borrie would meet with general acceptance. But why

is the proportion so low? It appears to be even lower than in the normal population. Is it a question of an alternation between a true depressive or other psychotic state and what are commonly regarded as psychosomatic conditions? This seems to me to be so in regard to prurigo and depressive states in middle age I have been struck by this sequence in patients coming into or leaving, mental hospitals, where they have been treated for depression. Is it generally accepted that the development of a psychotic shuts the door to psychosomatic means of expression?

RUSSELL DAVIS. The literature does lead one to the conclusion that the incidence of psychosomatic skin disorders is unusually low in mental hospital populations, and this seems to be the impression of those who have spoken about it above. I do not think anybody could suggest very clearly why it should be so It is possible that psychosis and skin manifestations are alternative ways of dealing with stress. Certainly one does see skin disorders clearing up when a psychosis develops, and perhaps more often the contrary a skin disorder appearing when a psychosis recovers.

DR S F WHITEHEAD (Cambridge). In discussing the specificity of stressors in symptom production, I have been impressed by a number of child patients in whom the apparent initial stress has not been either obviously specific or psychological, but where the parental attitudes and the patients attitudes towards the illness, have produced psychological stresses which seem to play an important part in the prolongation of the symptoms and in the moulding of the personality I suggest that these factors make it difficult when we try to discuss specific skin diseases in terms of primary psychogenic disorders such as the neuroses and psychoses.

WHITLOCK. I would like to comment briefly on the relationship of psychosis to psychosomatic disease as the subject is a controversial one. It has been said that the psychosomatic disorder protects the individual from psychotic breakdown and it has been observed that psychosomatic illness does sometimes interchange with psychotic symptoms. Ross (1954) has carried out a statistical study of this problem and Thomas, Stern & Lilienfeld (1956) also examined the matter Neither set of investigators was able to support the thesis that psychoses preclude a psychosomatic illness nor that psychosomatic disorders warded off psychosis. The problem, however is by no means settled.

ACKNER. I would like to support what Dr Whitlock has said and to some extent to take issue with Dr Russell Davis who regards psychosomatic disorders as alternate ways of dealing with stress. I think that this is reading psychological purposefulness into visceral symptoms which is not justified. I think we are quite justified in reading psychological purposeful ness into our neuro-muscular system which is under our voluntary control.

I could develop an hysterical paralysis and convince myself that I cannot write an examination because my arm is paralysed. My arm is under my voluntary control and that which I can control voluntarily I can use unconsciously for psychological gain. However there are systems which are not under voluntary control. Take, for example, the case of a young lady on her honeymoon who has *menorrhagia all the time. Perhaps emotional* disturbance caused the menorrhagia, but it would be improper to claim that the menorrhagia had occurred for the purpose of preventing sexual intercourse. This is to give the uterus a sort of independent psychological function as though it was a thinking organ and could decide what to do. It neglects the fact that the autonomic and endocrine system is in the way The old aphorism the sorrow that hath no vent in tears makes other organs weep is very applicable to the studies of Kepecs on exudation into the skin. It is not, however valid to claim other organs weep to stop us crying !

DR T B FITZPATRICK (Harvard) Would the speakers like to comment on the possibility that atopic dermatitis may be fundamentally an inherited metabolic defect and that the various precipitating factors simply aggravate this defect? Has anyone studied the urinary amino acid excretion pattern in atopic dermatitis? For example, abnormal tryptophane metabolites have been found in the urine of patients with scleroderma following tryptophane loading

WHITLOCK. *I agree that we need to know much more* about the biochemistry of these disorders. It is because of biochemical leanings that I became interested in the problem of central itching and its possible mediation by altered cholinesterase metabolism.

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COMPARATIVE MEDICINE

STICKINESS OF THE COAT OF GUINEA PIGS AN INHERITED DISORDER

A PRELIMINARY COMMUNICATION*

By B. M. HERBERTSON M. E. SKINNER
AND J A. H. TATCHELL

In 1955 a few of the guinea pigs bred in the Department of Pathology in Cambridge were found to have an abnormal coat. The affected animals appeared dishevelled and grubby To the touch their fur was sticky and felt as if a resinous material had been applied. As the sticky quality of the coat was the most impressive feature of the disorder the animals were called sticky guinea-pigs

The first guinea-pigs noticed to have this defect were the offspring of normal parents and had one or more normal sibs. The afflicted animals were born with a very sticky coat and, although from their appearance they seemed to be ailing this impression was misleading for they thrived and were as robust as their normal littermates. During the first few weeks the stickiness became less pronounced, but nevertheless persisted throughout life and was always readily recognizable. The defect did not appear to favour skin infections. Although normal and sticky animals have lived together for long periods in the same cages and have been given the same food no guinea pig born with a normal coat has become sticky This combination of features suggests that the condition is probably inherited and is not due to environmental factors.

This communication summarizes the results of breeding experiments and of microscopical and chemical techniques used in an attempt to discover the nature of the disorder

RESULTS

1 *Genetical*

Preliminary breeding experiments showed that stickiness is inherited. Outcross, intercross, backcross and homozygous sticky matings (241 off spring bred) have demonstrated that it is due to a single recessive factor with full penetrance and no appreciable effect on viability

* A more extensive account has been submitted for publication in the *Journal of Genetics*.

2. *Microscopical*

(a) *Hair* Although more debris is present on sticky hair than usual its structure appears normal.

(b) *Skin* No defect has been found in the skin of sticky guinea-pigs. The epidermis and dermis show no abnormality and the number size, structure and fat content of the hair follicles and sebaceous glands appear normal.

(c) *Organs and other tissues.* No defect of the major organs and tissues has been found.

3 *Chemical*

As the hair of sticky guinea-pigs loses its stickiness when washed with lipid solvents, but remains sticky when washed with water it seemed likely that a fatty substance present on the hair is responsible. Chemical investigation has shown that about four times as much fat can be extracted from sticky hair as from normal hair Analysis of the fat from normal hair has revealed that approximately one third of the lipid is saponifiable and two-thirds non-saponifiable. With sticky hair on the other hand, these proportions are almost reversed. In addition, the fatty acid fraction from sticky hair is more fully saturated than that from normal hair

A more detailed investigation of the lipids present on the hair and in the skin and elsewhere is being undertaken.

CONCLUSION

The stickiness of the coat of sticky guinea-pigs is due to the presence of an excessive amount of lipid on the hair Genetical experiments show that the defect is determined by a single recessive factor

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SCRAPIE A NERVOUS DISEASE OF SHEEP CHARACTERIZED BY PRURITUS

By A. C. PALMER

Scrapie is unique among the transmissible diseases of man and animals, unique because of the nature of the transmissible agent, length of incubation period and character of the histopathology Although the condition is primarily of importance to the veterinarian, and is not communicable to man, its study poses many fundamental problems concerning the genesis of disease. Because cutaneous irritation is an important clinical sign of scrapie it is hoped that this short review will be of particular interest to dermatologists.

CLINICAL SIGNS

Scrapie occurs naturally in sheep mainly in certain breeds. Animals show signs of intense irritation of the skin, abnormal gait, hyperexcitability tremor and progressive debility Fasciculation and nystagmus have also been reported (Palmer 1957*b*). Death can follow within a few weeks of the onset of signs or it may be delayed for many months. Skin irritation is usually severe animals rub themselves incessantly nibble the skin and tear out the fleece. All areas of the body surface appear to be affected, but wool loss is confined to regions that are accessible, notably the neck, flank and perineum (Pl. 1 fig 1). Rubbing the back manually often evokes the nibbling response in which the animal elevates the head, nibbles, salivates profusely and pushes its back vigorously on to the stimulus this behaviour suggests *that rubbing provides satisfaction.*

HISTOPATHOLOGY

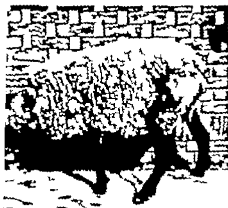
As the disease has such profound effects upon the animal, one might expect to find a clear-cut pathology However no macroscopic post mortem changes consistently occur There are secondary changes, such as abrasions of the skin and fatty metamorphosis in the liver Microscopically there is usually an intense vacuolation of nerve cells in the brain and spinal cord, which Besnoit & Morel first reported in 1898. Since that time the significance of this observation has enjoyed a variable fortune. There has been controversy as to whether the vacuolation is artifact and whether it has any association with the disease. However there is now much evidence to show that vacuolation is indeed not an artifact and that in animals suffering

from scrapie there are usually many more vacuolated neurones than are found in the normal (Bertrand, Carré & Lucam, 1937 Brownlee, 1940 Holman & Pattison, 1943 Palmer 1957*a*, *b* 1958 Zlotnik, 1957*b* 1958). Certain nuclear masses in the cord, medulla, pons and midbrain are particularly affected and the lesions are generally bilaterally distributed. In the cell, small vacuoles appear in the cytoplasm (Pl. 1 fig 2), there is loss of Nissl substance and the nucleus occupies an eccentric position. The vacuoles become progressively larger and may reach 80–100μ in diameter (Pl. 1 fig 3) sometimes there are several vacuoles in one neurone. An attempt to classify the morphological appearance of the vacuolation was published by Zlotnik (1958). Spheroidal eosinophilic bodies often occupy the vacuoles (Pl. 1 fig 4) and this material probably consists of degenerative products. Histochemically the material gives a positive coupled tetrazonium reaction indicating the presence of protein tests for nucleic acids are negative (Palmer 1957*b*). In addition to these intravacuolar bodies, Palmer (1957*a*) described similar material within the cytoplasm of apparently non-vacuolated neurones, but this observation was disputed by Zlotnik (1957*a*). In all probability the intracytoplasmic bodies observed by Palmer are intravacuolar bodies which appear to be within the cytoplasm on occasions when a vacuole is cut tangentially There is no evidence that the material represents a true inclusion body

NEURO ANATOMICAL DISTRIBUTION OF VACUOLATED NERVE CELLS

The selective distribution of vacuolated neurones in the central nervous system in sheep affected with scrapie has been reported by Holman & Pattison (1943) Palmer (1958) and Zlotnik (1958). Certain nuclear masses, namely the lateral cuneate nucleus, the lateral part of the reticular formation and the papillioform nucleus are often severely affected, while other nuclear groups in the cord and medulla remain normal. There may be a neuro-anatomical relationship between the clinical signs of the disease and the distribution of the lesions, but this must remain a matter for conjecture because so little is known about neuro-physiology of the sheep Involvement of the lateral cuneate nucleus may lead to inco-ordination but at present there is no explanation of the severe cutaneous irritation. Peripheral nerves, dorsal root ganglia and peripheral nerve terminations have been examined (Brownlee, 1940 Palmer 1957*b*) but all these organs appear to be quite normal.

PLATE I



Fig



Fig

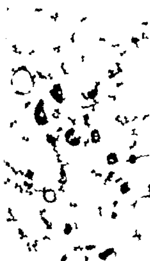


Fig. 3

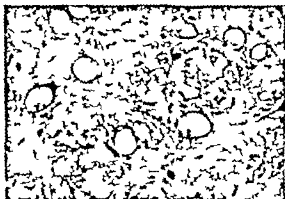


Fig. 4

For explanation see p. 244

(*Facing p. 240*)

EXPERIMENTAL TRANSMISSION

In 1936 scrapie was accidentally transmitted to normal sheep by the parenteral introduction of formalinized louping ill vaccine. This vaccine had been prepared from the brain of a sheep that had in fact been in contact with animals affected with scrapie (Gordon, 1946). Since that time several successful transmission experiments have been reported (Wilson, Anderson & Smith, 1950 Gordon, 1957 Stamp 1958). Many attempts have been made to find out the nature of the transmissible agent. When brain or spleen material from an affected sheep is injected into normal sheep, by the subcutaneous, intramuscular or intracerebral route, up to 60 per cent of animals may become affected after an incubation period varying from 4 months to 2 years. Moreover the agent can be passaged in series (Wilson *et al.* 1950). However the resistance of the agent to formalin, to boiling for 30 min. (Stamp) and to other viricidal procedures is quite unlike the resistance of a virus. As no laboratory animals have been found to be susceptible to the disease and because of the long incubation period in sheep work on the nature of the agent has been seriously handicapped.

In 1939 Cuillé & Chelle reported the transmission of scrapie to goats. This work was successfully repeated by Pattison (1957) and in 1957 Gordon & Pattison showed that in sixty-seven experiments on goats, there were 100 per cent takes. Moreover brain material from affected goats when inoculated into normal sheep caused the disease. Histologically changes in the nerve cells of the goat are similar to those found in sheep (Pattison) (see Pl. 1 fig. 4). This transmission of scrapie to goats, animals that do not normally suffer from the disease, provides additional evidence that some kind of infectious agent is involved. Nevertheless, there is also evidence that in sheep there is a factor responsible for congenital predisposition to the disease.

SIGNIFICANCE OF LESIONS IN SKELETAL MUSCLE

McGowan (1918), when investigating the condition in sheep suggested that the cause of the skin irritation was the presence of sarcosporidia in muscles. This idea was refuted by McFadyean (1918*b*) who showed that sarcocysts often occur in muscle from normal sheep that do not show signs of scrapie. In 1956 attention was again focused on muscle as Bosanquet, Daniel & Parry reported myopathic changes in sheep affected with scrapie and suggested that scrapie might be comparable to dermatomyositis in man. Bosanquet *et al.* did not consider vacuolation of neurones in the brain to be significant. Support for this hypothesis was given by Delez, Gustafson & Luttrell (1957) who found muscular dystrophy in three sheep affected with

scrapie. Attempts to confirm these observations have failed. Palmer (1957*b*) discovered a total of only three muscles from nine affected animals showing small lesions similar to those described by Bosanquet *et al.* No myopathy was reported by Gordon (1957) in sheep and goats in which the disease was experimentally produced. Work by Hulland (1958) in which the muscles from thirty cases of scrapie were carefully examined and compared with muscles from twelve control sheep showed that scrapie cannot be considered a primary disease of muscle. It therefore seems likely that lesions of myopathy can co-exist with scrapie, but are not responsible for clinical manifestations.

DISCUSSION

In the study of any disease process it is necessary to arrive at a satisfactory definition of the clinical and pathological entity. Although there is still some controversy about the variations of clinical manifestations in scrapie (variations that may arise as a result of secondary changes such as starvation) the clinical criteria do appear to have been fairly well established. The presence of a large number of vacuolated neurones in selected regions of the medulla can be regarded as confirmatory evidence.

The pathogenesis of scrapie is a major problem. Intense neuronal vacuolation cannot be disregarded. Vacuolation may be the final cytological manifestation of a local biochemical abnormality. On the other hand, it is possible that the nerve cell is the target of an abnormal metabolite liberated elsewhere in the body. In this connection medullas from sheep affected with a number of diseases other than scrapie were examined by Holman & Pattison and by Palmer (1957*b*): significant vacuolation did not occur in these animals.

Evidence for the experimental transmission of scrapie to normal animals can no longer be disputed, but it would be unwise to ignore the possibility of a congenital factor that leads to a predisposition for contracting the disease. Determination of the nature of the transmissible agent is severely handicapped because laboratory animals are not susceptible. Perhaps the use of tissue culture techniques may prove profitable. But what could be the nature of this agent, an agent that can resist so many biological insults? That it consists entirely of protein is unlikely in view of its stability to heat and formalin. There may be a non protein moiety, perhaps carbohydrate which on introduction into the body forms a template for the subsequent reduplication of the agent. But theories such as this must be put to the test and with scrapie this entails many months of tedious trials. If the nature of the agent causing scrapie can be finally determined the results may lead to spectacular changes in the present-day concept of the genesis of disease

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATE

PLATE 1

Fig. 1. A field case of scrapie showing loss of wool from neck and hind-leg.

Fig. 2. Early vacuolation from medulla of sheep affected with scrapie. There is loss of Nissl substance and the nucleus is in an eccentric position. Note the presence of small vacuoles. H. and E. ×800.

Fig. 3. Large vacuolated nerve cells in the lateral caudal part of the reticular formation. Compare affected cells with normal neurones. Thionin. ×175.

Fig. 4. Intense vacuolation in the caudal part of the reticular formation from goat suffering from scrapie. Eosinophilic material is present in several of the vacuoles: some of the latter are polylocular. H. and E. ×400.

IMMUNOLOGY

IMMUNE PHENOMENON IN RELATION TO THE SKIN

By R. R. A. COOMBS

I wish freely to admit that my interest in the skin as an organ was really aroused, for the first time, when I was asked to give this talk. Gathering together my thoughts has been a most valuable experience and, if it can prove of interest to you also I will be most gratified.

What I have tried to do is to construct an essay reflecting at one and the same time the interests of immunologists in the skin and those aspects of immunology which I have imagined would be of interest to those practising dermatology With this in mind I have approached the subject from three aspects (1) antigens of the skin (2) antibodies (3) antigen antibody reactions in the skin.

The discussion must, of necessity be cursory and, perhaps for this reason, it might be a *little unsatisfying for the specialist.*

ANTIGENS OF THE SKIN

A consideration of the antigens of the skin is a good illustration of one of the trends of immunology today Up till recently immunologists had devoted the greater part of their time to the study of the antigenic structure of micro-organisms. Now there is an ever-increasing interest in the anti genic structure of cells, tissues and organs of animals and man himself. One can think of such studies as serological anatomy The serologist, making use of his specific antisera, can reveal the antigenic substances or molecular building stones which go to make up the gross structures observed by the anatomist. The ability to recognize tissue and organ specific antigens should afford an invaluable tool for problems of ontogeny differentiation, structure and function. Further the increasing number of known intra-species antigens allows one to observe the individual's physical imprint on this cellular architecture. Besides the biological interest, serological anatomy is of obvious importance for pathology for how can the diseased state be studied without a sound knowledge of the normal?

Presumably many of the large molecular substances of the skin, that is, substances potentially antigenic, are not peculiar to the skin and its appen dages. Some, such as keratin, are confined to the skin, while the poly saccharides and proteins of the dermis, such as collagen, are not. Again,

anyone having a specialized interest in the antigens of the skin could not disregard, for instance, the antigens in vessel walls for such, although not essentially skin antigens, do form a vital part of its structure.

Organ and tissue specific antigens

There are two very characteristic proteins in the skin—keratin and collagen. With both these proteins the fact of their insolubility in the natural condition is presumably the main reason which has precluded extensive serological study. If a protein is insoluble, how do you introduce it into an animal so that it will stimulate antibody formation and how do you measure the antibodies which might be produced?

With keratin an opening was made in 1934 when Goddard & Michaelis found that on treating wool with alkaline thioglycolate a solution of keratin could be effected with apparently no other change in the protein than that of splitting open the disulphide bonds by conversion to sulphydryl groups. Pillemer, Ecker & Wells (1939), making use of this soluble form of keratin, or kerateine as they called the reduced form, showed that such keratin prepared from feathers, wool and human hair was antigenic in rabbits and showed definite species specificity. They also showed that the specificity depended to some extent on the redox state of the sulphydryl groupings of the molecule.

With collagen, a further difficulty is that of the purity of any preparation. However, a recent study by Watson, Rothbard & Vanamee (1954) has succeeded in providing good evidence of the antigenicity of rat collagen. Their antisera, produced in rabbits, also showed species specificity. These workers used, as antigen, collagen made soluble in dilute acetic acid. They showed that addition of normal rabbit serum or normal saline to this collagen solubilized in dilute acetic acid precipitated out fibres with the characteristic striations of collagen, but on addition of their rabbit anti-collagen serum there was no fibre formation but only a globular precipitate.

With specific antisera to both keratin and collagen it should be possible to make use of Coons's elegant immuno-histological technique (see Coons, 1956) using fluorescent antibody to get pertinent evidence on the question of collagen fibrilogenesis and on the development of keratin.

While discussing the fluorescent antibody technique of Coons, I should mention the recent work of Cruickshank & Hill (1953) who by means of this method and with a serum made against kidney glomeruli, were able to show what appeared to be a common antigen in epithelial basement membrane and reticulin. Their work indicated that this antigen was absent from collagen, cartilage and elastic tissue. Scott (1957) pursuing this work

further produced evidence that reticulin and basement membrane were in fact antigenically distinct.

Besides the scleroproteins there are also globular plasma-type proteins in the skin. This is over and above the true plasma proteins which are obviously to be found both intra and extra vascularly in the skin. Neuberger (1957) discusses the evidence for albumin-like proteins being synthesized by certain cells locally in the skin. This is a type of problem that could be answered with Coons's immuno-histological fluorescent staining technique.

Whatever their physiological significance, these albumin like proteins of the skin have another quite different importance from the medical point of view for within this group of globular proteins is to be found the main allergen of horse dandruff and presumably of other dandruffs also Squire in 1950 had concluded that the allergenic component in horse dandruff although distinct from serum albumin, showed an immunological cross reaction with this serum protein and suggested this as the explanation of the severe reactions experienced by some horse sensitive asthmatics when first injected with therapeutic horse serum.

In recent years the gel diffusion technique has been revolutionizing this type of antigenic analysis, and Stanworth in 1957 has been able to demonstrate by this method up to seven antigens in crude dandruff extracts. The allergenic fraction, as far as man was concerned, was a main (B_4F) glycoprotein which had an electrophoretic mobility different from that of horse serum albumin. Stanworth concluded that the serological relationship between horse dandruff and horse serum proteins was due to the admixture of small amounts of serum albumin (and globulin) with the horse dandruff antigens and was not the result of any cross reaction between the two types of protein.

From this slight excursion we must return to consider other large molecular weight substances in the skin such as the muco-polysaccharides of the ground substance. Quinn & Cerroni (1957) and Quinn & Singh (1957) failed, however to produce antibodies in rabbits to chondroitin sulphate made from human costal cartilage or to hyaluronic acid from human umbilical cord. According to Glynn, Holborow & Johnson (1956) their earlier suggestive results using the mucopolysaccharide, chondroitin sulphate adsorbed on streptococcal vaccine, were due to contaminating blood group substances (also mucopolysaccharides) in the preparations studied.

One of the inducements for investigating the antigenicity or haptenogenicity of the mucopolysaccharides of the ground substance has been the idea that there may be some auto-immune phenomenon occurring in the ground substance in rheumatic fever (Glynn, 1958).

Individuality antigens

Up till now we have been considering tissue or organ antigens common to all animals of the species. Individuality antigens are those, such as the blood group antigens on red blood cells, which vary from one individual to another within the species and are part of the physical basis of individuality. Those persons primarily interested in skin might well ask if any of these so-called individuality antigens are to be found on skin cells. My colleagues and myself (Coombs, Bedford & Rouillard, 1956) were able to answer this in the affirmative, as by means of the mixed agglutination reaction we were able to show the ABO blood-group antigens on squamous epidermal cells—the antigen corresponding to that of the blood group of the person. Unfortunately we were not able to show the Rh antigens on these cells (Ashhurst, Bedford, Coombs & Rouillard, 1956).

To indicate the way serological individuality is going I should say that now not only are there well over fifty individuality red-cell antigens, but also, quite distinct from these, individuality antigens on white cells and platelets. And what is more, recent works by Oudin (1956), Dray & Young (1958) and Dubiski (1958) show there to be individuality antigens within the group of plasma proteins.

Antigens responsible for homograft rejection

The experimental work on homograft rejection over the last fifteen years, mainly emanating from the laboratory of Medawar and his colleagues, Billingham and Brent, point to its being an immunological phenomenon —that is, the process of rejection being an actively acquired immunological response. For reasons both obvious and perhaps less obvious to the non-specialist, skin has been the tissue or organ which has allowed of the best analysis and yielded the most significant advances.

In attempting to summarize briefly the present state of our knowledge on the process underlying the rejection of skin homografts, the following points can be made (see Brent, 1958). Transplantation immunity depends upon the presence in the tissues of the donor of antigens absent in the host. These antigens are governed by the so-called histo-compatibility genes. In identical twins a graft from one to the other takes permanently. No circulating antibody responsible for the rejection of a skin homograft, has, so far been demonstrated *in vitro*. Brent, Brown & Medawar (1958) invoke the delayed type hypersensitivity reaction as the means by which the graft is rejected.

Concerning the antigens responsible, these appear to be quite distinct from the known blood-group antigens. They appear to be common to all

nucleated cells of the body—skin and other organs—and so show no organ specificity. The antigens have been shown to come from the nucleus and not from the cytoplasm. They had been thought to reside in the nucleoprotein fraction, but now the evidence suggests that they are mucoid substances with the nucleoprotein possibly acting as an adjuvant (Medawar 1958).

These then are further antigens to be found in skin cells as well as in other nucleated cells of the body. From the point of view of this essay I want, above all, to emphasize the vital role skin has played in unravelling the mechanism of transplantation immunity.

Auto-immunization

A knowledge of the antigens present in the skin is a desirable prerequisite for any investigation into the possibility of auto-immunization involving antigens of the skin. The clinical concept of auto-sensitization to skin is being discussed by Mr Parish and the one or two general points I wish to make may perhaps serve as an introduction.

First, there can no longer be any doubt that auto-immunization in the strictest sense may occur either under physiological control or as a pathological process following the breakdown of some controlling mechanism. A discussion of the controlling mechanisms would, I am afraid, take a lecture in itself, involving as it does a full consideration of Burnet's self marker theory, the concept of immune tolerance, the natural habitat or location of the various antigenic materials in the body and the question of whether they ever, under normal circumstances, enter into the environment of antibody producing cells. To avoid ambiguity and misunderstanding definitions need to be very precise.

Secondly, what I want to stress is that, despite much suggestive evidence from both the clinic and laboratory, there are still very few definitely proven examples of auto-antibodies as such and, especially, of auto-antibodies playing any significant role in the pathogenesis of a particular disease. What proof should be sought? Here I would like to bring before you certain postulates, which have become known as Witebsky's postulates (Witebsky *et al.* 1957), which, ideally, should be fulfilled in order to prove the role of an auto-antibody in the pathogenesis of a particular disease:

(1) the direct demonstration of free circulating antibodies that are active at body temperature or of cell-bound antibodies by indirect means;

(2) the recognition of the specific antigen against which this antibody is directed;

(3) the production of antibodies against the same antigen in experimental animals;

(4) the appearance of pathological changes in the corresponding tissues of an actively sensitized experimental animal that are basically similar to those in the human or animal disease under study

These requirements have come close to fulfilment in certain cases of human chronic thyroiditis (Witebsky *et al.* 1957) and should prove most helpful to other workers attempting to implicate auto-antibodies in the pathogenesis of any particular disease.

ANTIBODIES

Antibodies are produced by plasma cells and possibly by other lymphoid cells as well. As far as we know there are no essentially skin-made antibodies, although of course antibodies could be produced locally in the skin by a settlement of plasma and lymphoid cells in that area (White, Coons & Connolly 1955). It follows that the remarks I am going to make hold for antibodies in general.

The last thing I wish to do is to minimize the great complexity of types and serological behaviour of antibodies, but such detailed considerations would be out of place here and really must be left to the specialists.

Broadly speaking, antibodies—whether they be hetero- iso- or auto-antibodies—fall within one of three categories

(1) Circulating plasma antibodies of the ordinary immune type detectable *in vitro* by at least one of a variety of methods.

(2) Circulating plasma antibodies which have a special affinity for passively sensitizing tissue cells. These may or may not be demonstrable *in vitro*. (The Prausnitz–Küstner antibody comes into this category.)

(3) Postulated antibody like substances within lymphoid cells responsible for the delayed type of hypersensitivity reactions. These have not been shown *in vitro* nor characterized.

When the serologist produces antisera for the recognition and characterization of antigens, it is usually antibodies of category (1) that he uses because their reactions can be studied in the test tube. Antibodies falling within the second category that is those able passively to sensitize tissue cells, are very important clinically because of the role they play in sensitization reactions of the immediate type. This division between categories (1) and (2) may be artificial and it is probably nearer the truth to say that, of the circulating plasma antibodies, some give an *in vitro* reaction and apparently lack the capacity passively to sensitize tissue cells—some antibodies possess both these characteristics while again some are specially endowed for passively sensitizing tissue cells and at present elude detection *in vitro* in the absence of living cells. That part of the immune response which we have divided off into category (3) may play a considerable part in moulding

the pathology of a disease and may be used for diagnostic purposes as in the tuberculin test.

It is, however important to realise that the full immune response of an individual to antigenic stimuli works through all three channels, although certainly at times, the main reaction may be canalized through any one of these channels to the partial exclusion of the others. For instance, in man, the antibodies resulting from ordinary parenteral injection of antigens fall mainly within category (1)—these are the antibodies about which we have a fairly satisfactory body of knowledge. In hay fever asthma and other urticarial or immediate-type sensitivities antibodies in category (2) are mainly involved, while in contact dermatitis and seemingly the homograft reaction (Brent *et al.* 1958) antibodies in category (3) play the major role.

I think there can be no doubt that, up to the present, the main stumbling block to full liaison and collaboration between the dermatologist and immunologist has been the inability of the latter to detect, measure and characterize in the laboratory the Prausnitz–Küstner type of antibody (category (2)) and the postulated antibody like substance responsible for delayed type hypersensitivity reactions (category (3)). When this can be done we can optimistically forecast a most fruitful period of co-operation between the clinic and the laboratory

Claims to measure the Prausnitz–Küstner antibody *in vitro* and in the absence of cells, have of course been made and a recent one by Gordon, Rose & Sehon (1958) deserves special attention.

Here we must leave the discussion on antibodies, but in doing so I do not want to gloss over our ignorance as to the mechanism of passive sensitization of tissues and cells. We do not know for certain, what cells are involved nor the nature of the affinity between the antibody molecules and cell membrane. These gaps in our knowledge are made very obvious when contrasted with the well-documented facts supplied by the pharmacologist on the *active substances released by these reactions and their subsequent* effects.

ANTIGEN–ANTIBODY REACTIONS IN THE SKIN

Before mentioning the well recognized skin hypersensitivity reactions mediated by antigen acting on cell-bound antibodies there are other immune phenomena which take place in the skin and which merit brief note.

Toxin–antitoxin reactions in the skin

First, there are toxins such as the erythrogenic or scarlatinal toxins of group A haemolytic streptococci which cause the skin rash in scarlet fever According to van Heyningen (1950) the mode of action of the toxins is not

understood but their effect on the skin can be neutralized by homologous antitoxin. This neutralization can be shown by a specific blanching of the erythema and is made use of in the Schultz–Charlton blanching test for scarlet fever. Along the same lines and more important is the Römer method of diphtheria antitoxin titration. This is based on the specific prevention of the swelling and oedema produced in the abdominal skin of the guinea pig by very small doses of toxin.

Immune cytotoxic action in the skin

Another immunological situation in the skin which can be envisaged as a lesion produced by cytotoxic action initiated by the direct combination of antibody with some susceptible tissue component of the skin. A good, if somewhat experimental, example of this is afforded by the essentially haemorrhagic and necrotic lesion produced in the skin of the guinea-pig on injection of a small amount of rabbit anti Forssman serum. Redfern (1926) explained this local haemorrhage and necrotic reaction, as well as the generalized haemorrhagic shock produced by larger injections intravenously as a direct cytotoxic action of the antibody on guinea-pig tissue containing Forssman antigen. This explanation is further strengthened by the recent work of Tanaka & Leduc (1956) who showed, using Coons's fluorescent antibody technique, that in the guinea-pig the Forssman antigen is present in the endothelium and adventitia of blood vessels.

Purpura due to sedormid sensitization

A somewhat similar type of reaction, more germane to general dermatologic practice, underlies the generalized purpura following administration of sedormid in a sensitized patient. Ackroyd (1958) has shown that sedormid forms a loose association with platelets and, also possibly with certain endothelial elements. Some persons produce ordinary immune antibodies (category (1)) against these complexes. The antibodies react back against the drug platelet complex (or drug-endothelium complex) and cause the destruction of these cellular elements with the ensuing purpuric condition. This example of drug hypersensitivity would appear to be essentially a cytotoxic reaction affecting platelets and possibly also, although the evidence is not so strong, vascular endothelium—an essential component in the skin.

Arthus phenomenon

If one injects antigen into the skin of rabbits which have circulating precipitating antibody there occurs swelling, erythema and induration. Depending on the severity of the reaction this may go on to necrosis and ulceration. The process is *initiated* not by a reaction of antigen on passively

sensitized cells as in passive cutaneous anaphylaxis or as in the immediate wheal type reaction in tissues sensitized with the Prausnitz–Küstner antibody but essentially by a micro-precipitation of circulating (category (1)) antibody within and about the blood vessels. The white-formed elements of the blood become adherent to the immune aggregates and cause thrombosis. At the same time the damaged cells liberate pharmacologically active substances which further increase the permeability of, and damage to, the vascular system. There is an intense perivascular infiltration of polymorphs. The thrombi which form lead to haemorrhage, infarction and subsequent necrosis.

No reaction occurs in animals where the circulation has been temporarily depleted of polymorphonuclear leucocytes (Humphrey 1955) The phenomenon is not inhibited by anti-histamines. This skin lesion is very easily produced in the rabbit and may be an undesirable complication in rabbits being repeatedly injected for antisera production. Really as an uncomplicated entity it is rather an artificial and experimental syndrome and, for this reason, should not be seen as such in man. Cases, however have been reported in the older literature following repeated injections of therapeutic sera. On the other hand, this type of reaction may be involved to some degree or other in some of those diseases of obscure aetiology but with a suggestive allergic or immunological background.

Before leaving this reaction I would like to stress two things. First, that these reactions, as observed under anything but the strictest experimental conditions, seldom represent a single defined entity or process (Gell, 1958), and secondly that such reactions as these vary very greatly in the different animal species and man.

Immediate wheal or urticarial-type skin reaction

I really need say very little about this reaction as it is shown *par excellence* by human skin and is very familiar to all clinicians. The localized wheal or more generalized urticaria is directly referable to histamine and like substances liberated by cells passively sensitized with the Prausnitz–Küstner antibody (category (2)) in the presence of antigen. Besides the short-lived inter and intra-cellular oedema there is very little reaction to be seen histologically I have already mentioned the great need for laboratory methods to measure this Prausnitz–Küstner type of antibody which, at the moment, can only be shown reliably by passive transfer tests in man himself Once we have laboratory tests the way will be open to study which cells are involved and the mechanism of passive sensitization itself.

Delayed-type hypersensitivity reactions

This type of host response, in contrast to the immediate reaction, is totally cellular in character and takes up to 48 hr to reach its full intensity. The distinctive hallmark of the reaction is the slow infiltration of the area with mononuclear cells which gives the reaction its delayed character.

A typical example, of course, of a delayed type of hypersensitivity reaction is the classic tuberculin reaction. A similar mechanism is, apparently also at work in contact dermatitis and in the host's reaction to homografts (Brent *et al.* 1958).

The evidence seems clear that the postulated antibody like substance or specific factor responsible for this reaction is carried by lymphoid cells. The nature of this substance and how to measure it *in vitro* elude us at present. When we have this knowledge we will be in a much better position to understand the delayed reaction itself.

Significant factors in the initiation of this delayed type immune response in contact dermatitis are, first, the special reactivities of the proteins of the skin with chemical haptens (Eisen, Orris & Belman, 1952 Eisen & Belman, 1953), secondly the influence of the nature of these protein carriers on antibody production (Mayer 1957) and thirdly the localization of the coupled antigenic substances in the skin.

This introductory survey has of necessity been very sketchy. I have not attempted to analyse and translate the various clinical conditions such as allergic urticaria, atopic dermatitis, allergic eczema and the fixed reactions into the classic prototype reactions which I have mentioned. Nor have I considered the special immune or allergic responses characterizing certain anti-bacterial inflammatory reactions in the skin. I have, of course, been limited by lack of personal experience and also by the desire to confine my remarks to basic principles.

It would not be possible, however to conclude this discussion without referring briefly again to the so-called collagen diseases (Klemperer, Pollack & Baehr 1942 Klemperer 1954). Whatever the essential causes of these diseases are eventually found to be, there can be no doubt that they have many immunological aspects associated with them. One can cite (i) the factor in the serum of patients with rheumatoid arthritis responsible for the Rose-Waller test (ii) the antibody like factor in such sera which Grubb (1956) has shown is capable of differentiating serologically two distinct genetic types of human γ-globulin—Gm-positive and Gm-negative—and (iii) the factor in the serum of patients with lupus erythematosus which has all the characteristics of an auto-antibody to the DNA

of the cell nucleus (Miescher 1957 Seligmann, 1958). These are surely indications enough of some very basic immunological involvement.

The skin then, as you can see, offers much of interest to the immunologist. I am sure you can, in part, sympathize if this interest at times becomes somewhat esoteric and a little divorced from the problems presenting in the clinic. The immunologist's excuse is that his primary purpose must be the building up of the body of knowledge constituting his science. But I think you will agree with me that his reward is much greater and his work the more justified, if the knowledge gained can help the dermatologist and physician to alleviate the suffering of their patients.

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AUTOSENSITIZATION TO SKIN

By W. E. PARISH

The concept of autosensitization was introduced by Whitfield (1921, 1922, 1931) to account for the occurrence of a generalized erythemato-urticarial eruption of the skin about 10 days following local trauma of intact skin, or a local area affected by dermatitis.

Since that time there have been a number of descriptions of generalized skin reactions following various forms of eczema. Brown (1939) described such generalized reactions as a sequel to a variety of forms of eczema. Smith (1945) under the title of 'Eczema autolytica' observed an exacerbation of a primary, usually chronic, localized dermatitis concomitant with a generalized erythema, developing papules and vesicles in certain predilection sites. He believed that a toxic blood-borne lysin was responsible, which had its origin as a foreign protein derived from bacteria or a tissue breakdown product sensitizing the patient to his own exudate. The absorption of some poison from the affected area producing a generalized cutaneous intoxication has also been proposed by Whitfield (1926). Hopkins & Burky (1944) refer to a number of cases of eczema, particularly chronic eczema of the hands, in which it is believed that staphylococcal toxins have initiated the autosensitization to skin, and claim to have produced improvement or cure in their patients by the use of staphylococcal toxin. In their series of over 50 patients *Staphylococcus aureus* was recovered from the cutaneous lesions of forty and *S. albus* from only two of their total number. Templeton, Lunsford & Allington (1949) described five cases of autosensitization to skin: one apparently initiated by drugs and one by bacteria, the other three were idiopathic in origin. Haxthausen (1955) found that eighty-eight of 235 cases of varicose eczema developed a generalized vesicular eruption with a distribution similar to that described by Smith and Young (1958) in a summary of sixty cases, also noted that exacerbation of an initial localized lesion may precede the generalized eruption. One other worker (Milbradt, 1935) in a vague description and without considering the phenomenon of autosensitization, did suggest making use of the anti-allergic property of extracts of skin and liver in the treatment of erythroderma.

DEFINITION

Autosensitization is the sensitization of the body by circulating antibody or a delayed cellular-type reaction, or both, to constituents of its own tissues.

The body does not normally become sensitized to its own tissues, and for this to occur the tissue must be presented to the body in an abnormal form. In the skin the abnormality may be a sequel to tissue degeneration where proteins in various stages of denaturation are present at the same instant, and over a period of time antibody formation becomes progressively modified to include some of the normal components of skin. In the majority of cases, part of this process may be the sensitization of the body to extraneous material which may include bacteria or fungi and their toxins, foreign organic debris from the environment, or certain tissue reactive chemicals in a number of household products or therapeutic dressings. These may form protein–hapten complexes with the tissue components, with the end result that antibodies with skin specificity are formed.

To make an accurate diagnosis of autosensitization it is essential to demonstrate that circulating antibodies or cellular sensitivity exists specific for the body tissues, and if this autosensitization is believed to be the cause of disease it should be possible to demonstrate that this sensitization will produce a cytotoxic or other effect resulting in lesions identical with those of the disease.

Careful clinical observations indicate that autosensitization to skin does occur but the laboratory proof of this phenomenon is still lacking.

AUTOSENSITIZATION TO DANDER

It has been demonstrated by Storm van Leeuwen et al (1922, 1923, 1926, 1929), Keller (1924), Murphy & Cobe (1932), Hampton & Cooke (1941), Simon (1944–8), Cormia & Esplin (1950), Esplin & Cormia (1951) and Lipschultz (1953) that certain individuals may produce an immediate or delayed type of reaction on being skin tested with human dander or crude extracts of dander and it has been postulated that the dander is an important potentiating factor in the development of the eczema. Most of these tests have been carried out using homologous material, but Simon (1947) in one case and Cormia & Esplin in all their cases using autologous material have demonstrated that patients may be sensitive to products of their own skin.

There is little doubt that some dander products may be naturally antigenic, for Hampton & Cooke and Simon (1944, 1947, 1948b) demonstrated that passive sensitization of normal human skin was possible with

the sera of some patients giving positive direct skin tests, and the sera could be neutralized *in vitro* by dander extract. Hampton & Cooke went further by demonstrating that the sensitizing antibodies were of the reagin type, that is, there were no specific precipitins demonstrable in the test sera, and the sensitizing capacity was destroyed by heating at 56 C. for 4 hr The skin sensitizing titre of these sera was described as high, which from their earlier observations was about 1/1000. They also demonstrated that cross neutralization of these sera was possible using house dust as antigen, thus confirming the earlier results (Hampton & Stull, 1940) obtained by the use of the Schultz Dale technique but relatively large amounts of house dust were required and they therefore suggested that the cross neutralization was due to the small amounts of human dander present in the dust. Simon (1944) was unable to demonstrate cross neutralization with a larger number of products. On the other hand, Lipschultz was able to demonstrate precipitating antibodies to the dander antigen in nearly every case in which it produced a positive skin reaction in the sixteen patients tested.

If we described dander as consisting of shed epithelial cells with the degenerating products of sebum containing house dust, bacteria, fungi and possibly the residua of cosmetic preparations it is not unlikely that some of the products will be antigenic to hypersensitive patients. Fungi are known to be antigenic and several species of fungi cross react, as demonstrated by the work of Jadassohn, Schaaf & Wohler (1937) and a further review by Jaros & Kirsner (1948). Therefore homologous skin material used for testing need not contain the same fungal organism for the reaction to be elicited in a sensitive patient. Furthermore, contamination of skin material by fungi may give the appearance of *autosensitization*. Simon's (1948*a*) fourteenth case, a woman sensitive to her own dander has much in common with the first case described by Hughes & Hamilton (1958) where the woman was sensitive to *Pityrosporum ovale* present in her own scalp

Simon (1944) failed to demonstrate sensitivity to certain micro-organisms including *staphylococci* and *Pityrosporum ovale* in five patients by direct skin testing or passive transfer tests with their sera. But Lipschultz was of the opinion that bacteria, particularly *Staphylococcus albus* were responsible for the sensitization in his series of patients though it is difficult to reconcile the statement that coagulase-negative *S albus per se* is not antigenic to a person with autosensitization when tested intradermally in view of his own results in Part A of his experiments. In this part of his work 93 per cent (i.e. fourteen) of sixteen patients gave a positive reaction to a heat killed suspension of an *in vitro* growth of organisms from an affected area, and there could be no question of carry-over of contaminating skin antigen. It is also difficult to accept the further statement that incubation and

conjugation (of the bacteria) with other substances are apparently required to produce detectable antigenic power as the same sixteen patients treated with an incubated mixture of homologous skin scrapings and bacteria also gave a 93 per cent positive response. His assertions are indeed true when applied to another twelve patients tested with *S. albus* from the skin of one normal person in which he obtained a 25 per cent positive direct skin test, and after the organisms and skin scrapings had been incubated together prior to the injection, 50 per cent of the twelve patients gave an immediate type response yet only one of twenty control normal individuals gave a reaction.

To be certain of a diagnosis of autosensitization *sensu stricto* it is essential to demonstrate that antibodies or a cellular sensitivity exists specific to the body's own protein or tissue constituents. The use of skin material for testing obtained from a stillborn infant (Simon, 1944, 1948*b*) certainly reduces but does not entirely eliminate the possibility of extraneous contamination but the suggested prior sterilization of the dander extracts before use would be of little avail, for loss of viability of any micro-organisms is no safeguard that their antigenic components will be destroyed, especially if these micro-organisms are intimately associated with the host cells. Thus any denaturation of their protein would be accompanied by a similar destruction of host tissue protein, and moreover polysaccharides and certain waxes remain unchanged during many of the normal sterilization procedures.

A serious potential source of error in direct skin testing is the hypersensitivity of the skin of some individuals to the test substances independent of any antigen-antibody reaction. Hampton & Cooke with dialysed dander extracts and Simon (1944, 1945) using untreated extracts, discovered that the dander extracts could be irritant to normal individuals. Confirmatory evidence has been obtained by the author who affected by no known sensitivity has found that certain preparations of his own dander and that of one other person of the same blood group could be primary irritants even after prolonged dialysis against buffered saline. Untreated extracts could contain a histamine like substance indeed Murphy & Cobe (1931) observed that there was a close similarity between the reaction obtained by dander extract and certain dilutions of histamine. However Storm van Leeuwen, Bien & Varekamp (1923), Keller (1924) and Hampton & Cooke (1941) dialysed their test extracts, and the last named tested the final product by the Shultz Dale technique to demonstrate that it was free of histamine type factors.

Such treatment only reduces and does not eliminate all possible causes of a non-specific reaction. It is well known that individuals with an atopic type of eczema will give a positive skin reaction on being tested with a

large number of unrelated compounds, as is recorded in several of the case histories of Hampton & Cooke (1941) and Simon (1948*a*) and there appears to be little doubt that the skin of some individuals may give a positive response to materials to which they are not immunologically sensitized. Thus an abnormal individual may react to the presence of material which is non irritant to the normal subject.

The nature of the antigen is an additional source of confusion. Hampton & Cooke used dander from normal scalps, as did Simon in many of his experiments, but he (1947 1948*b*) also demonstrated that it existed in dander from the general body surface though in lower concentration. In further experiments Simon (1948*a*) gained evidence that eczema scales, in particular those from one patient, produced positive skin tests in other individuals, but not to the person from whom they were taken. Furthermore, patch tests on twenty-four patients and controls performed simultaneously indicated that the active principles of human dander and the eczema scales were not identical. In their work Cormia & Esplin believed that they found a water soluble antigen in epidermal scales which was the potent factor but this was not confirmed by Lipschultz who demonstrated that, using extracts from homologous skin scales, those from the affected area gave a 75 per cent response in sixteen patients, those from an area adjacent to the primary site a 62 per cent response, and normal scales taken from an area well removed from the lesions only gave 19 per cent (i.e. three individuals) positive skin tests, which was regarded as supporting evidence for his contention that skin antigens alone were not the cause of the sensitization.

In a continuation of the work on these lines, Cormia & Esplin in both their papers, and Lipschultz attempted to demonstrate that autologous circulating white cells contained antigen, because intradermal injection of suspensions of these cells, or plasma, produced a positive reaction in several of their tests, some of which were a delayed type of response.

Gross amounts of antigen would, however have to be present before circulating white cells would be sufficiently saturated to produce a specific skin test response, *even if taken from a vein draining the area. If such a* response were due to a sensitization there is more probability of it being the result of introducing a concentrated suspension of immunologically active cells into an area containing antigen. This would have some similarity to the skin transplantation immunity studies in guinea pigs as described by Brent, Brown & Medawar (1958) in which cells expressed from regional lymph nodes of guinea pigs hypersensitive to homografts, injected intra dermally into the donor animals resulted in a delayed or tuberculin-type inflammatory response.

But nearly all the positive reactions on skin testing with leucocytes described by these workers were of the immediate type, and examination of the methods they used in their isolation of the cells from the blood unfortunately makes no mention of any controls to determine the presence of histamine like factors released on withdrawing the blood. The methods necessary to prevent undue release of histamine on taking blood samples have been described by Humphrey & Jaques (1954) and in an experiment by the author it was found that blood withdrawn into an anticoagulant, kept cool and handled gently during centrifugation, may still have a variable concentration of some smooth muscle contracting substance in the plasma when tested on guinea pig ileum in the Schultz Dale bath, and pure platelet suspensions would have *to be washed in a large volume* at least three times before they were free of histamine-like activity. In the experiments under review there were platelets still present in the leucocyte suspension (Cormia & Esplin, 1950) and human platelets contain histamine, 5-hydroxytryptamine and an unidentified third substance (Humphrey & Jaques, 1954). It is generally recognized that individuals with atopic eczema develop an abnormal response to the injection of histamine, and this may be one reason for the difference in response between eczematous subjects and controls to the injection of crude leucocyte suspensions.

It would appear from a consideration of the data available at the present time that some form of sensitization to shed epidermal cells may occur but whether it takes the form of a true autosensitization to the skin tissue is unknown, and until the antigen or its complex is identified it is too early to express any valid opinion. In many of the cases described as autosensitization *the sensitivity may be directed against the* microflora of the skin. Nevertheless, the recognition that human dander may be a factor in the exacerbation of an existing eczema, no matter in what way it may act, has an important clinical application.

EXPERIMENTAL EVIDENCE IN SUPPORT OF THE CONCEPT OF AUTOSENSITIZATION TO SKIN

Whitfield (1921) described three distinct conditions in which autosensitization to skin may have taken place. In the first extravasation of the patient's blood beneath the intact surface of the skin following local trauma, resulted in a generalized erythematous urticarial eruption 10 days later. He believed the eruption to be due to absorption of the broken down products at the site of the injury to which the patient became sensitized. Some support for this theory is gained from the work of Landsteiner & Jacobs (1936) who demonstrated that an acyl-protein conjugate derived from guinea-pig sera

was a very potent antigen for testing the skin sensitivity of guinea-pigs sensitized previously by application of the acyl-chlorides. However more significant confirmatory evidence is obtained from the experiments of Bizzozero (1936) who in nine of twenty five persons obtained a tuberculoid nodular or papular reaction following repeated intracutaneous injection of the individual's own fresh serum in the same area of the arm, and after regression of the nodule the reaction could be elicited by merely rubbing the area without further injection. The sensitivity persisted for any time up to 20–45 days after the last reaction, and injection of serum near to the original area resulted in a similar but weaker response. Injection of the patient's whole blood resulted in an immediate wheal followed by a nodule, this wheal, however may have been due to histamine release from damaged blood cell elements. Thus there is some evidence that degeneration of serum proteins in the presence of skin tissue may result in a sensitization of the skin.

Whitfield's second description where exacerbation of a persistent eczematous dermatitis of the leg was followed 11 days later by a generalized haemorrhagic papular eruption, is the model which concerns those interested in the cause of idiopathic generalized eczema and the experiments to investigate its nature. Templeton *et al.* (1949) induced precipitin formation in five normal persons using an extract of homologous skin, but this gives little evidence for the susceptibility of man to autosensitization. Homologous skin is a foreign material as demonstrated by the rejection of homografts, and which may contain incompatible blood-group antigens (Coombs, Bedford & Rouillard, 1956).

Hecht, Sulzberger & Weil (1943) were only able to demonstrate the presence of precipitating antibodies to homologous skin in rabbits with certainty by the intramuscular injection of a gross amount of skin (20 ml. with adjuvant) and repeated intracutaneous injection of staphylococcal toxin. It is significant that the antigen prepared to detect the serum precipitins was obtained from pieces of skin that were not bacteriologically sterile, and the skin had to be incubated at least 24 hr before the antigen was present in sufficient concentration. Thus the precipitating antibodies were detected by antigen obtained from degenerate skin.

In other experiments on rabbits Voisin & Maurer (1955) discovered that homografts implanted on six animals that had been previously injected intramuscularly with skin from the donor rabbits and adjuvant, developed abnormally Subsequently four of the six test rabbits rejected autografts, which the authors attributed to autosensitization to skin. Attempts to demonstrate sensitivity by intradermal injections of extracts of skin produced no response.

Rosenthal, Baer & Hagel (1958) were unable to sensitize guinea-pigs to

autologous skin when the skin emulsion and adjuvant, and in some cases staphylococcal toxin, was injected by the intracutaneous, intramuscular or intraperitoneal routes. These guinea pigs accepted autografts and showed no abnormal response on being subjected to skin trauma, and the procedure of encouraging an in vivo degeneration of the reinjected skin by using fresh material would more closely simulate the changes taking place in a dermatitis, than the in vitro incubation of the skin prior to injection carried out by other workers.

It would appear that even using homologous skin material growth abnormal conditions have to be imposed in order to achieve sensitization in the majority of cases, and the attempt to induce autosensitization to skin in the guinea pig did not result in one case of sensitization out of eighty-nine animals tested. Though there are very variable species differences to all forms of sensitization, autosensitization to skin should be regarded as a task not lightly to be undertaken by the body.

The third form of autosensitization dermatitis described by Whitfield (1921) was the fluid liberated from vesico-bullous eczema lesions giving rise to urticarial wheals and vesicles on the skin in the vicinity of the lesion, whereas the same fluid failed to give a reaction on Whitfield's own arm. For this observation there is less confirmatory experimental evidence. Cormia & Esplin found that the fluid from blisters raised on the skin of patients with eczema by the use of cantharides plasters for 24 hr. were primary irritants. A similar response is also said to occur in the Urbach-Königstein's technique for passive transfer of sensitivity in allergic contact dermatitis using fluid obtained by the use of cantharides blisters (Urbach, 1924 and brief review by Skog, 1955). This is of interest in the light of Milgrom's recent report (Milgrom, Wozniczko & Dudziak, 1957) that a small blister raised by light friction contained, for the first 3 hr., fluid with an antibody that would agglutinate all human red cells including those of the individual, and it was believed that this antibody was anti-O. But after 24 hr. the anti-O antibody was weak, and the strongest blood-group antibody was that normally found in the individual's own serum. Whether or not this is an antibody and not a non-specific inflammatory factor, it is possible that there may be other antibodies or cytotoxic factors liberated from skin under abnormal physiological conditions.

EXPERIMENTAL

Autosensitization to the skin of the pig

During a series of investigations carried out on skin diseases of pigs, the details of which will be published later, a study of the degeneration of the skin was made in a chronic exudative dermatitis that has some features in

common with human eczema. It mainly affects young pigs and may be due to some dietary or intestinal disorder though the real cause is unknown. Histologically there is marked acanthosis of the stratum Malpighii and inter and intra-cellular oedema, a variable degree of parakeratosis, and in the advanced cases hyperkeratosis with deposits of coagulated serous material on the surface which in the living animal becomes coated with dirt. Apart from the gross fissures of the stratum corneum in the very advanced cases there is relatively little evidence of bacterial infection.

Affected and normal pieces of skin were taken from the same pigs, cleaned of all bristle and the outer layers of the stratum corneum, dissected free of fat, extracted in saline and dialysed. These extracts were used as antigen to coat tanned red cells to determine the presence of agglutinating antibodies in the serum of the test animal, and in the sera of normal pigs and other pigs with dermatitis. A total of 303 sera from pigs with various forms of dermatitis were tested of which six, all of them cases of chronic dermatitis of the young pig, were found to have agglutinating antibody in titres between 1/8 to 1/128 to extracts of their own skin. In four of these cases the antibodies would agglutinate cells coated with extracts of autologous affected and normal skin. In the remaining two the antibodies were directed against components of the affected skin only. These antibodies to the same, or lesser extent would agglutinate cells coated with homologous affected or normal skin extract. *The livers and spleens taken fresh at post-mortem examination* from three of the affected pigs and seven normal pigs were extracted in the same way and used to coat the tanned red cells, but no specific agglutination resulted to either the autologous or the homologous extracts.

Further tests demonstrated that the antibody could be neutralized by addition of extracts of affected and normal skin in the sera of the four pigs agglutinating both, and by affected skin extracts in the other two. Extracts of homologous skin could neutralize or partially neutralize the antibody. Two of the affected pigs were give a series of injections with a laboratory strain of *Escherichia coli* at the completion of the preliminary tests demonstrating the presence of skin auto-antibodies, and the sera were tested to determine the agglutinating antibody titre to the somatic antigen. Other samples of the same two sera were then absorbed with fresh cut sections of autologous skin and then tested for the presence of the anti-skin antibody by tanned cell agglutination and the agglutinating titre to the *E. coli*. It was found that the skin antibodies were completely removed and the bacterial antibodies left intact.

Sera from 216 normal pigs contained no antibody for the individual animal's own skin, nor agglutinating antibody for tanned red cells coated with affected homologous skin.

There is some evidence that not all agglutinating antibodies have the power to sensitize tissues. Therefore passive sensitization tests were carried out on normal pigs, in which the skin was injected intracutaneously with 0·2 ml. serum and later challenged by extracts of affected skin. The controls consisted of sera containing agglutinating antibody challenged by extracts of normal skin, and two injections of 0·2 ml. normal pig serum, one being challenged by an extract of normal skin, the other by affected skin. The recipient animals had never received injections before and neither the donor nor the recipient contained serum antibodies capable of agglutinating the other animal's trypsinized red cells, though erythrocytes were only obtainable in four of the animals with auto-antibodies to skin, as the other two were no longer available for study at the time of these tests. No attempt was made to test for the presence of any serum antibodies to the circulating white cells. The passive sensitization tests were repeated with the same results, and the extracts and sera demonstrated to be free of histamine-type activity by tests on guinea pig ileum in the Schultz Dale bath.

All six test sera produced an immediate type erythematous reaction to affected skin four test sera, two of which contained no agglutinating antibody to normal skin were able to sensitize skin passively to extracts of normal skin. Intracutaneous injection of the two sera with the highest antibody titre was followed by a local faint erythematous reaction which was far more marked on subsequent challenge with the extract of skin. Thus it appears that the antibody to normal skin was directed against some component within the cell, and not readily accessible in the intact cell.

Intravenous injection of the serum containing antibody into one normal pig and another with chronic dermatitis, had no effect on the former and appeared to make the latter worse 24 hr later but there is no scientific proof of this.

Serum containing this skin auto-antibody was centrifuged and filtered through a 0·4µ filter and incorporated in the media used for the in vitro growth of explants of regional lymph nodes taken fresh at post-mortem examination of the affected pigs. Growth of the explants was retarded when the autologous serum was used in the media and definite morphological differences resulted, but not when homologous normal serum was used. No such changes were noted in explants from the spleen. It is regretted that no blood white cell counts were made of the affected animals.

Incubation of freshly excised homologous skin in the diluted sera containing skin auto-antibodies and normal sera, resulted in morphological changes in both when compared with the control tissue that had been immediately fixed. Though definite differences existed between skin exposed to normal sera and that containing antibody chief of which were

the greater loss of alkaline phosphatase activity in the cells containing it and some diffusion of the remaining enzyme, variable vacuolation of the cytoplasm and a marked reduction in the capacity of the mitochondria to metabolize Janus Green B.

The antigen extracted from the skin, particularly the affected areas, was water soluble, but on repeated freezing and thawing from −20 C. was precipitated in an insoluble form which also occurred on long storage at −20 C. with only a single thawing, with loss of antigenicity in the supernate. It was precipitated out completely with 1 per cent trichlor acetic acid, destroyed at 100 C. for 5 min. and did not pass a dialysis membrane. Its nature is unknown, but it appears to be a protein.

Bacteriological examination of the skins of the six sensitized pigs revealed a small range of species only on the skin surface or in extracts. None of the pigs showed antibodies to these organisms as judged by agglutination and conglutinating complement absorption tests on the sera. The frozen and thawed extracts of the bacteria used to coat tanned red cells revealed no agglutination and the same extracts did not inhibit the tanned cell test for anti-pig skin auto-antibody Injection of these organisms into normal pigs *did not reproduce the disease. There were no significant fungi.*

It cannot yet be claimed that autosensitization to skin in these pigs is definitely proved, but antibodies have been found in these animals specific for their own skin, for it is unlikely that in the volume of material extracted as antigen that the tanned cells would selectively adsorb some protein foreign to pig skin. Though these antibodies have been demonstrated to have a cytotoxic effect *in vitro*, there is no evidence that they play any important part in perpetuating the disease neither has it been determined whether there exists a cellular sensitivity to the autologous skin tissue independent of circulating antibodies.

Investigations on autosensitization in man

Experiments were started to determine if autosensitization to skin was detectable in man by similar techniques. With the kind help of Dr Rook and Dr Champion twenty five sera have been taken from selected cases of eczema and tested by the tanned cell test using homologous normal and affected skin as antigen and in one case autologous skin. So far no serum antibodies to skin have been discovered.

However further examination was made of the spontaneous precipitates of the freshly prepared saline skin extracts, which were initially believed to be the result of the lower solubility of the protein at +4 C. than at room temperature, and continued autolysis, but I am indebted to Dr Coombs for the suggestion that these precipitates may also contain antigen-antibody

antibodies is the phenomenon of immunological paralysis. Felton (1949) and several others demonstrated that large repeated injections of polysaccharides into normal mice would inhibit antibody formation to these antigens, and that antibodies could not be stimulated a long time afterwards by injection of amounts capable of inducing antibodies in the normal animal. The duration of this inhibition, however, is still uncertain, for Baer, Bringaze & McNamee (1954) were unable to confirm Felton's results that it would persist for three months after a single large dose of antigen. Large doses of proteins do not produce immunological paralysis in the normal animal capable of producing antibodies (Dixon & Maurer, 1955a) but they do greatly retard the detectable presence of antibodies, presumably because the surplus antigen must be removed before free antibody can be released. If the proteins of normal skin were antigenic, the vast amount of material would either prevent antibody formation or immediately mop up the antibody, unless they were in some generally inaccessible position whereby enough was liberated to be antigenic but the rest protected from the immediate presence of antibody.

Experimental evidence is still required to determine the mechanism of the oft repeated tests whereby homologous skin scale extracts will elicit a reaction on intradermal injection of the sensitized person, yet when the serum from the sensitized person is injected intradermally in a normal individual no reaction is reported unless the site is challenged by the dander extract. Thus the antigen is either confined to the outer layers of the skin which have avoided contamination on injection, or as suggested on the results obtained from investigations in the pig, is confined to constituents within the cells and not readily available within the intact cell. If this be so, then there would be insufficient available antigen *in situ* to neutralize the antibody, or unite with it in the presence of living cells to give a spontaneous reaction at the site of passive transfer, and the process would continue as in similar tests using human serum containing antibody to foreign protein.

Should the autosensitization to skin be a sequel to inflammation, as many of the clinical reports suggest, then it is possible that a variable degree of the antibody formation may occur locally at the inflammatory focus. De Gara & Angevine (1943), Oakley (1953) among many others have demonstrated that local antibody formation in the skin of the rabbit may follow intradermal injection of antigen, in the majority of cases at a time when little or no antibody was detectable in the regional lymph nodes. Though it is still necessary to prove, no matter how unlikely the contingency, that the inflammatory focus has not become the site of concentration of the first formed antibody produced elsewhere. In the presence of a chronic

inflammatory focus in the skin there is little doubt that there will be a certain amount of subclinical physiological deterioration of skin in most areas, and if this reflects the same pattern of degeneration as the original focus there will be potential antigenic sites to remove circulating antibody wherever formed, and if a cytotoxic affect results it could give rise to further lesions. This would obviate the suggestion that it was necessary for antigenic material to be released from the primary site and to become bound to other areas of the skin providing generalized specific antigen sites of abnormal skin protein.

For the onset of the delayed type of sensitivity prolonged contact or presence of antigen may not be required, for several workers including Landsteiner & Chase (1939) and Schnitzer (1941 1942) have sensitized an area of skin of guinea pigs and excised that area any time from 12 hr to 4 days later and demonstrated that generalized dermal sensitization has still followed.

Investigations on the rejection of homografts provide some relevant information, but it must not be assumed that the phenomena of resistance to homografts and autosensitization to skin are identical, though there is evidence that autosensitization to skin may manifest itself by rejection of autografts.

Medawar (1956) states that if any antibody does exist in animals sensitized to a homograft they are not sufficient to account for the reaction, and it seems certain that they are not γ-globulins. But neither is it known with any certainty whether the circulating antibodies demonstrated in those cases described as autosensitization to skin in man play any significant part, or whether they are just a concomitant process to a cellular sensitivity Recently Mackay Larkin & Burnet (1957) demonstrated another possible modification in the auto-immune antibody as found in the serum, in which auto-immune antibody from two patients failed to react with liver spleen and striated muscle antigens prepared from the individual s own tissues when tested by the complement fixation test, but could react in high titre when tested against the same organ antigens obtained from other individuals. Furthermore the organ antigens of these two patients were capable of fixing complement in the presence of sera from other individuals with the same disease, that is, macroglobulinaemia or disseminated lupus erythematosus. They suggested that these results could indicate that abnormal circulating antibodies capable of reacting with the individual's own tissues may be absorbed out in the spleen or elsewhere, leaving its heterologous acting This could be advanced as an explanation that individuals with skin sensitivity to homologous skin extracts fail to give a positive response on testing with autologous extracts. But our present limited knowledge of

the initial cause and the nature of the antigens concerned in these diseases provides no reliable evidence for this contention, for conditions of this nature may completely or partly be caused by a stimulus of external origin. It was once believed that the leucocyte agglutinins found in the sera of certain patients, *not subjected to certain drug treatment, which would* agglutinate many other individuals leucocytes, but never those of the patient whose serum was being examined were due to an autosensitization, until it was realized that they were the sequel to multiple blood transfusions (Payne, 1957 Dausset, 1958).

It is improbable that the complete antigenic similarity between leucocytes or lymph node cells and cells of the skin graft demonstrated by Medawar (1946 1956) in the homograft reaction would have any comparable importance in the process of autosensitization The homograft antigens are principally individuality factors which are not applicable to tissues within the same individual. Therefore the discovery that pig skin auto-antibodies produced consistent and repeatable morphological effects on the explants of regional lymph nodes and not in explants of the spleen, all obtained from the same animals is suggestive of the presence of some antigen associated with the lymph node and probably drained from the skin tissue, that was not present within the splenic tissue.

Without overlooking the differences existing between the phenomena of homografting and autosensitization, the mechanism of the abolition of tolerance described by Billingham, Brent & Medawar (1956) could have some bearing on autosensitization if the sensitivity could be set up without a profound alteration in the skin. It was demonstrated that normal acceptable lymph node cells taken from a mouse of one strain, and injected into another of the same strain tolerant to a homograft, resulted eventually in a sensitivity to that homograft. If a clone of immunologically active cells could arise in a normal individual, that failed to recognize an antigen that was only intermittently or in low acceptable concentration introduced to the body as possibly in the fully differentiated cells of the stratum corneum, and this clone of cells managed to persist in the body then it is conceivable that autosensitization could result without the initiation of a marked change in the constituents of the skin. However at the present moment this remains unfounded speculation.

Summary of tests to demonstrate autosensitization to skin

There are three possible series of events that may produce the clinical appearance of autosensitization to skin.

(1) A sequel to inflammation when the body may become sensitized to its own skin tissue

(2) Sensitization to dander products of unknown specificity and usually associated with the reagin type of antibody

(3) Sensitization to micro-organisms and their products contaminating the skin. This is not autosensitization unless sensitization to skin tissues coexists.

The intradermal injection of autologous skin or dander extracts is beset with so many possible sources of error that it is an extremely unreliable test which may give many false positive results, or as demonstrated in experimental work, may fail to give a response when autosensitization to skin apparently exists.

Agglutinating or precipitating type antibodies may be demonstrated without difficulty in the serum of the individual, provided the test antigen is free of any significant contamination. In those cases in which no circulating antibody is demonstrable the antibody may still be present bound to the skin tissue and would have to be eluted. Then it would be essential to demonstrate not only the presence of an active principle but that it was an antibody

Those individuals affected by the so-called atopic eczema appear to have a reagin type antibody in the serum, and this can only be demonstrated reliably by passive transfer tests on normal individuals with all the attendant dangers.

The two most significant tests, though probably less adaptable to the requirements of medical practice, are the response of the individual to local test trauma, and autografts. Those individuals who have, or have been affected by eczema, and who are subjected to localized trauma on an area of normal skin sufficient to cause marked hyperaemia, which subsequently develops eczemal lesions similar to those present elsewhere, are probably sensitized to their own skin, but this finding alone cannot be accepted as positive proof Normally individuals will accept grafts of their own skin, and the repeated rejection of normal autologous skin from normal prepared sites may be accepted as an indication of autosensitization. In every case the aim should be concentrated on demonstrating the presence of circulating antibody or cellular sensitivity to known constituents of the skin.

Unfortunately it is evident that there are many factors involved in the onset of eczema that lie outside the realm of immunology Not all papular or vesicular eruptions will be the result of antigen-antibody reactions, and not all cases of a generalized eczema following exacerbation of a local area of dermatitis will be a sequel to autosensitization to skin.

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MECHANISMS OF DERMAL HYPERSENSITIVITY

By P G H. GELL

A number of different kinds of allergic lesions, which are to a greater or lesser extent analogous to various lesions of human disease can be produced in experimental animals. Such lesions are differentiated primarily upon the basis of the presence or absence of antibody Those which depend upon the presence of antibody and which can be provoked (at least in part) by the injection of anti-serum from an immunized into a normal animal, are the lesions of local anaphylaxis and the Arthus reaction

By and large, local anaphylaxis in the skin is equivalent to an injection of histamine, that is, it is evidenced by a local permeability of the capillaries and hence oedema, without any permanent damage. In the Arthus reaction, on the other hand (whose mechanism is essentially different from local anaphylaxis and which has been differentiated on the basis of a number of criteria (Benacerraf & Kabat, 1950) of which an important one is its failure to be suppressed by anti-histaminics (Fischel, 1947)) damage may be considerable and lasting The Arthus reaction is also essentially a lesion centred on the local blood vessels, in particular the venules—it is characterized (Rich & Follis, 1940 Stetson, 1951) by the appearance at the site of antigen injection in an immune animal of vascular thrombosis and consequent ischaemic necrosis. The Arthus reaction is in fact an infarct but it is characterized by the blockage not of one large vessel but of all the small vessels in the dermis. The thrombosis is the result of the accumulation in the blood vessels at the site of white cells and platelets—in the later stages, presumably as the result of necrosis, the periphery of the infarct is invaded by vast numbers of polymorphonuclear leucocytes but the primary injury is vascular This sort of lesion necessarily takes some time to develop, and the Arthus reaction reaches its peak at 2–4 hr Hence the local anaphylactic reaction (which takes 10 min.) may be called an immediate reaction and the Arthus an early reaction

Reactions which occur in the absence of demonstrable antibody take even longer to reach their maximum from 6 to 24 or in humans even 48 hr they are delayed reactions Important reactions which fall under this head are the tuberculin and the other reactions of bacterial hypersensitivity such as those to certain streptococcal antigens. The lesions of experimental contact sensitivity analogous, in some respects, to human

contact dermatitis belong here—so also in all probability do the reactions of homograft rejection (Medawar 1958 Brent, Brown & Medawar 1958) though this has recently been questioned (Gorer 1956 Stetson & Demopoulus, 1958) In none of these are antibodies apparently relevant to or directly concerned in the reaction, though in all these cases antibodies of some sort are present. In tuberculin-positive animals for instance, antibodies regularly occur against numerous antigens of the tubercle bacillus but it has been shown again and again that their presence or absence is not in any way correlated with the presence or absence of classical tuberculin sensitivity Similarly in animals sensitized to contact with say picryl chloride antibodies are usually demonstrable against picryl proteins but they are not apparently relevant to the sensitivity as such. Animals may be passively injected with large amounts of 5 anti picryl-protein anti-serum so that they will in fact give strong Arthus reactions when injected intra dermally with picrylated protein from a different source but they possess no contact sensitivity *Similarly animals if tested early in the course of* sensitization with picryl chloride possess no circulating antibody against picryl (as evidenced by the absence of the anaphylactic state") but give positive contact reactions (Benacerraf & Gell to be published).

Delayed reactions are characterized by the infiltration into the tissue exposed to antigen of mononuclear cells, lymphocytes or histocytes and by the hypertrophy of local mesenchyme cells, including the cells lining the small blood vessels, the vascular endothelium. Sometimes this hyper trophy is so intense (if a large amount of antigen is used) and the cells crowd in in such numbers, that vascular damage and necrosis may occur but necrosis is not characteristic of delayed reactions and when it occurs one usually suspects the co-existence of an Arthus reaction caused by the presence of irrelevant antibodies against some components of an impure antigen.

A word may be said about the kind of antigens involved in such reactions. It used to be thought that full antigens, proteins such as egg albumin or the serum proteins of another species, stimulated only antibodies and hence antibody-dependent reactions, while odd substances like tuberculin (a large polypeptide) and certain other obscure bacterial products, only and always provoked delayed reactions. This is now known not to be the case recent work (Dienes & Schoenheit, 1929 Dienes & Simon, 1935 Freund & McDermott, 1942 Raffel 1948 Uhr Salvin & Pappenheimer 1957 Benacerraf & Gell, 1959), building on older foundations, has shown that any protein can be under appropriate conditions persuaded to provoke delayed reactivity at a time when no antibodies are demonstrable It is, however the case that denatured and partially hydrolysed proteins (Gell &

Benacerraf 1959) or very small doses of proteins normally precipitinogenic (Salvin, 1958), tend especially to provoke delayed sensitivity Moreover the so-called active Arthus reaction, in an immunized animal that is, is actually a combined reaction, containing thrombotic elements which are classically Arthus in type, with other cytological elements which derive from reactivity of a delayed kind (Gell & Hinde, 1954 Tremaine & Jeter 1955). Hence such experimental lesions are extremely difficult to interpret. Clearly lesions in humans which derive from active immunization of any kind— even auto-immunization as in thyroiditis will also tend to be of this complex character

The basic mechanism of each of these allergic lesions is different and in most cases still obscure. The best understood, that of anaphylaxis (both local and systemic), is a biochemical study which I am little qualified to discuss. Its end result is, however the release from damaged cells of hormones which vary in their nature, quantity and pharmacological effects from species to species histamine is still of dominant interest, but acetyl choline, serotonin, heparin, etc. are also involved. The effect, however is essentially pharmacological the hormones are released, exercise their effects, diffuse away or are destroyed, and the tissue returns to normal.

The basic lesion of the Arthus reaction is almost certainly at the vessel wall whether antigen is free in the tissue and antibody circulating in the blood, or antibody injected intradermally and antigen injected intravenously the result is the same. When the vessel wall is put into this abnormal state as a result of having become the battlefield of a precipitin reaction it shows various characteristics. The cells hypertrophy they also exhibit the property of phagocytosis which they do not normally possess (Gell, personal observation) they also evidently offer an irresistible attraction for circulating polymorphs and platelets. If one reduces the numbers of polymorphs to low levels with toxic materials (Humphrey 1955), or renders the blood entirely incoagulable with heparin (Benacerraf, 1953), the reaction is largely suppressed so evidently the effect on the vessel wall is not alone sufficient there must be formed elements to supply the physical materials for blockage.

The basic mechanism of delayed reactions is very obscure indeed. All one can say is that the animal is in such a state that certain of its cells move towards the site where antigen is deposited. Presumably these cells are the primary repository of sensitivity but there is no evidence, except analogical, that an antibody of any kind whether cell fixed or not, is involved. The mononuclear cells of the blood and the lymphoid system are sensitized in this sense, since they have been shown (Chase, 1953) to transfer delayed reactivity of various types on the whole the evidence (Cruickshank, 1951

Pepys, 1955; Waksman & Matoltsy, 1958) is against the idea, once prevalent, that all the mesenchyme cells of the body are actively sensitized. It is just possible that the vascular endothelial cells share in this specific sensitivity; on them there is no decisive evidence either way.

The mechanism of contact sensitivity is even less clear because here we are not even sure what the effective antigen is. In some cases, the contactant is known to react readily with proteins, and it is reasonable to suppose that a protein conjugate is formed by combination of the chemical with body proteins *in vivo* and that this then stimulates the state of delayed sensitivity. However, if this were the case, contact-sensitive animals without antibodies should react with a tuberculin-like reaction to intradermal injection of an artificially made conjugate (carefully freed of the simple chemical sensitizer); similarly, if delayed sensitivity to a protein-conjugate can be provoked by modern methods, the animals should also exhibit contact sensitivity to the simple chemical. Neither of these appears to be regularly the case (Benacerraf & Gell, to be published; Eisen, 1958), especially if adequate care is taken to remove contamination with the simple chemical, a vitally necessary precaution. Hence one must either suppose that the sensitizer combines with some special, homologous protein, or that the cells react to the simple sensitizer itself. This latter theory, though difficult to hold on chemical grounds, is more consistent with the fact that so many contact sensitizers do not combine with proteins and are in fact chemically inert.

Delayed sensitivity in general, is a kind of immune reaction which probably develops earlier in foetal life than antibody production, which though often less specific is more sensitive to small amounts of antigen, which develops much more quickly after immunization—in fact, has all the signs of a primitive type of reaction pattern. The phylogenetic incidence has never been investigated in the light of newer knowledge, such as it is; such a study would be of the greatest value in deepening our insight into its nature and function.

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TUBERCULIN HYPERSENSITIVITY

By R. A. BRUCE

In no other infection has the influence of an allergic state engendered so much discussion as in tuberculosis. In its original sense the word allergy denotes a particular reaction of the tissues of the host towards a specific antigen to which there has been previous exposure. Etymologically the word is derived from two Greek roots, ergia denoting reactivity and allos—altered. Von Pirquet (1906) in his original definition outlines the basic aspects of the allergic process, and he states that allergy is the acquired specific altered capacity to react. Acquired, implies a previous adequate exposure to the sensitizing agent or antigen; specific, refers to the chemical constitution of the antigen; and altered capacity to react, describes the various reactions elicited. Later on the term tuberculin hypersensitivity arose in medical terminology to distinguish tuberculin allergy from the other forms of allergic reactions, and also it came to be regarded as indicating a state of acquired resistance.

The allergic reactions encountered are of two main types. The immediate type, which includes anaphylaxis, the Arthus reaction, hay fever, asthma and urticaria; and the delayed type occurring as a result of sensitization to bacterial, fungal or chemical antigens. The delayed type of reaction develops slowly and shows a maximal response between 48 and 72 hr. The reaction depends upon the combination of antigen with antibody; and the passive transfer of this type of allergy by means of lymphoid cells from sensitized subjects has been reported by many workers both in animals and in man (Kirchheimer, 1947; Metaxas, 1948; Lawrence, 1949; Wesslen, 1952).

The intracutaneous reaction to tuberculin is an example of the delayed type of allergic reaction. The antigen is the tuberculin which is injected, and the antibody is derived from the sensitized lymphocytes in circulation. The tuberculin skin test, or more familiarly the Mantoux reaction, after the French physician Charles Mantoux, born in 1877, is of considerable value in clinical medicine both for the diagnosis and management of tuberculosis.

A measurement of the degree of tuberculin hypersensitivity can be made by injecting into the skin varying amounts of tuberculin in a constant volume, and comparing the reactions obtained. The conventional doses in common usage are 1, 10 and 100 tuberculin units in 0·1 ml. of fluid (1 unit equals 0·00002 mg. PPD). The degree of hypersensitivity present

may be so great as to produce reactions to all three doses or it may be so small as to produce no reaction to 1 and 10 units, and only showing its presence with 100 units. There are instances where there is apparent failure to react to all three doses. The intensity of the tuberculin reaction can be influenced by altering the availability or persistence of antigen at the site of inoculation, the source and availability of antibody or the ability of the tissues to respond with an allergic reaction to the local combination of antigen and antibody (Pepys, 1955). Of particular interest are those subjects who fail to react to 100 T U given in the conventional intracutaneous inoculation, but who can be shown to react to tuberculin if the persistence of the antigen can be increased by using a particular vehicle.

The incorporation of tuberculin in an emulsion of liquid paraffin and lanolin— depot tuberculin—for intracutaneous injection, results in increased potency (Seeberg, 1951 Pepys & James, 1956) and in prolonged local persistence, the latter being demonstrated by the appearance, after B C.G vaccination, of positive reactions at the site of previously negative depot tuberculin tests (James & Pepys, 1956) The oily vehicle is irritant if injected too superficially and more acceptable methods of preparation and application of depot tuberculin have therefore been sought. A recent paper (Pepys, Bruce & James, 1958) describes reactions to tuberculin incorporated in various ointments and pricked into the skin by multiple puncture with the apparatus described by Heaf (1951). This following report describes the results of skin tests using one of these tuberculin creams. This preparation has proved very sensitive, and free from the obvious disadvantages of the earlier depot tuberculin preparations of tuberculin in oil, requiring intracutaneous injection.

MATERIALS AND METHOD

The tuberculin employed for testing was purified protein derivative (PPD) of *Mycobacterium tuberculosis* H37Rv (Weybridge) For the preparation of a concentration of 15 mg PPD per ml. in aqueous solution for admixture with the ointment, $N/10$ NaOH was added drop by drop to powdered PPD which was made into a paste, and then diluted with sodium borate solution (0·25 per cent). This material was then mixed with Eucerin (ointment of wool alcohols B.P) to give a final concentration of PPD in the test ointment of approximately 5 mg PPD per gramme. The cream was stable and did not separate off on standing at room temperature.

The PPD cream was pricked into the skin with the Heaf gun. The tests were performed on the skin of the anterior aspect of the forearms. Positive reactions were seen after 72 hr., and in doubtful cases, light friction of the test sites made the reactions more clearly visible. The reactions corre

sponded with the Grade I of the conventional Heaf test, showing small discreet indurated papules at the puncture sites.

For the comparison of the potency of the PPD cream with the conventional Mantoux test, the subjects were tested with 1, 10 and 100 T.U. With the first two strengths, reactions of 5 mm. diameter or more of induration were regarded as positive, but with the 100 T.U. test a smaller palpable reaction was regarded as positive.

THE INVESTIGATION

The investigation fell into two parts

(1) The comparison of the potency of the cream with the conventional tuberculin skin test.

(2) The capacity of the cream to persist in the skin of negative reactors, and the ability to give positive reactions at a later date, after these subjects had been vaccinated with B.C.G. Since this reaction indicates the development of tuberculin allergy following the vaccination, it will be termed the conversion test.

THE RESULTS

The PPD cream was compared with the conventional tuberculin skin test in two groups. Group (*a*) thirty two probationer nurses, from eighteen to twenty years of age. (*b*) 489 factory employees, from seventeen to fifty years of age.

Table 1. *Group* (a) *total number of subjects* 32

Test material	Positive	Negative	Total
Mantoux			
1 T.U.		20	32
100 T.U.	3	7	10
PPD cream	5*	2	7

* One reaction doubtful.

Table 2. *Group* (b) *total number of subjects* 489

Test material	Positive	Negative	Total
Mantoux			
1 T.U.	382	107	489
100 T.U.	24	83	107
PPD cream	43	38	83

The depot cream gave positive reactions in all subjects who reacted to the Mantoux tests.

The subjects in group (*a*) who were negative to the 100 T.U. test but who reacted to the PPD cream were given a B.C.G. vaccination test for the study of the significance of the positive reaction to the cream.

These fifteen reactors to the PPD cream were given an intracutaneous injection into the outer aspect of the thigh of 0·1 ml. B.C.G vaccine. The vaccination sites were examined 3 7 and 21 days later

Subjects who are positive to tuberculin show an accelerated response to B C.G vaccination. The reactions were regarded as accelerated if there was early development of an inflammatory indurated papule the inflamma tion settling within 7 days and leaving residual induration at 3 weeks. In contrast, subjects who are completely negative to tuberculin develop a slowly progressive lesion from the twenty first day onwards, but show no initial reaction during the first 14 days. The lesion in these cases progresses to ulceration.

At 3 days after the B C.G vaccination accelerated reactions were present in twelve out of the fifteen nurses two subjects had doubtful reactions and one had no reaction at all.

At 7 days, fourteen out of the total fifteen had accelerated reactions. At 21 days fourteen out of the fifteen showed accelerated reactions which had subsided to leave a small indurated papule and in the case which had given a very doubtful PPD cream test an ulcer was present. The three subjects who had been positive to the 100 T U test were similarly vaccinated and each gave typical accelerated responses. In none of the B C.G vaccinated subjects was there any disturbing reaction either initially or later

The conversion test was carried out in the two negative reactors to PPD cream from group (*a*) and thirty of the thirty-eight negative reactors in group (*b*) making a total of thirty-two in this group Two implants of PPD cream were made, one punctured into each forearm the first test was given on one forearm at 3 weeks before vaccination (1), and the second on the other forearm on the same day as the vaccination (2). When the final readings were carried out at 6 weeks after vaccination, test (1) had been *in situ* for 9 weeks and test (2) for 6 weeks. In none of the thirty-two subjects was there an accelerated reaction at the site of the B C.G vaccina tion. At both PPD cream test sites, reaction of the Heaf Grade I type were fully developed at 6 weeks, and at the same time the B C.G reaction had progressed from papule at 3 weeks to ulceration in all cases at 6 weeks.

Table 3

Days after B.C.G vaccination	Positive at PPD site	Positive at PPD site	Appearance at B.C.G site
3 days			
7 days			•
days	8	8	All papules
42 days	3	32	All ulcers

DISCUSSION

This work demonstrates the enhanced potency of this PPD cream preparation when introduced into the skin with the Heaf gun, and also has served to reveal low degrees of sensitivity to tuberculin in subjects who fail to react to 100 T.U. That there must be some immunological relationship in this type of sensitivity to the *Mycobacterium tuberculosis* seems to be borne out by the accelerated reactions to the B.C.G. vaccine. Post B.C.G. Mantoux tests showed that the tuberculin sensitivity of these subjects was increased by vaccination. This indicates that their initial low degree of tuberculin sensitivity was not due to an inherent lack of capacity to react.

Accelerated reactions to live or dead B.C.G. vaccine in subjects who fail to react to tuberculin have been regarded as the expression of infra-tuberculin allergy by De Assis & Carvallo (1942). This problem has been comprehensively documented and discussed by Fourestier & Blacque Belair (1957), who are amongst the proponents of a double test, employing both tuberculin and B.C.G. vaccine.

The PPD cream test appears equally effective as the B.C.G. vaccine in demonstrating the presence of infra-tuberculin allergy. It has the virtue of not changing the immunity status of the subject as does the B.C.G. test. In the ordinary way those subjects who fail to react to 100 T.U. would be regarded as suitable for B.C.G. vaccination, but the PPD cream test shows that, included amongst these, there must be a number with this very low degree of sensitivity to tuberculin. By means of the PPD cream test it should be possible to investigate the immunological role, if any, of infra-tuberculin allergy in tuberculosis as compared with the protection conferred by B.C.G. vaccination in completely tuberculin negative subjects. The true significance of these very low degrees of tuberculin sensitivity in relation to immunity is an important problem, as yet unanswered and awaiting elucidation.

The high concentration of PPD empoyed in the cream test is not a non-specific irritant. It persists for many weeks, without reaction, in the skin of tuberculin negative subjects only to react when the B.C.G. vaccination has induced a state of tuberculin allergy.

SUMMARY

A single PPD cream test has in this investigation served to show the presence or absence of tuberculin sensitivity and also the conversion of tuberculin negative subjects after B.C.G. vaccination. The innocuity of

the reactions elicited make it possible to start testing with this material without carrying out successive Mantoux tests with increasing doses of tuberculin.

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DISCUSSION

Chairman DR C. D CALNAN (London)

DR D I MCCALLUM (Nottingham). In the demonstration, I was interested to note how similar the anatomy of the pig's skin was to that of the human. Is the histology of pig eczema identical with that in the human? For experimental work, can the pig's skin be sensitized relatively easily to chemicals? If so would it be practicable to apply such a sensitizer to an extensive area of skin and then either exercise the animal or heat the environment, in order to bring about a secondary sensitization eruption?

MR W E. PARISH (Cambridge). The skin of the pig has many histological and histochemical features in common with the skin of man and it is possible that the pig may be a valuable experimental animal for investigation of some skin conditions and other diseases of man. I have been fortunate here at Cambridge to be able to make a comparative study of several skin conditions in the pig and man, and though differences exist in all of them, it is probable that the cause as well as the response may have much in common.

The dermatitis of the young pig sometimes called an exudative epidermitis which I briefly described this morning has the histological feature of vesicle formation in its acute form. In the early phase there is an inter and intracellular oedema within the stratum Malpighii, and later vesicles are formed there and beneath the stratum corneum much resembling those found in eczema in man.

I regret that I do not know of any investigations on the experimental induction of sensitivity to chemicals in the pig's skin I also regret that I cannot give a definite answer concerning the effect of exercise on the pigs affected by the type of dermatitis described as opinions are conflicting but it appears that exercise does make the condition worse and that this change is reflected more within the thin skin of the abdomen, groin and axilla, than in other areas where the skin is naturally thicker

DR BRIAN RUSSELL (London). Mr Parish said that grossly abnormal conditions must be imposed on an animal's skin if autosensitization is to develop

In man, too, autolytic eczema may occur when the immediate external environment is greatly modified. It can result from over treatment with antiseptics, from mixed infections or even from occlusion under water repellent ointments, with resultant maceration and perhaps a warmer surface temperature. It might be that the moist, warm partially anaerobic

conditions encourage an abnormal flora and that these organisms could be the sensitizing agents.

PARISH. The evidence that grossly abnormal conditions have to be imposed in order to produce autosensitization to skin in experimental animals has already been presented, and in our limited experiments to date we have been unable to induce autosensitization to skin in the pig.

As far as bacteria are concerned I do not believe that they play any important part in the sensitization to skin in the condition studied in the pig, even though they may be involved in the aetiology of the disease itself. The importance of bacteria or fungi and their toxins in autosensitization in man is yet to be determined, but it is unlikely that all forms of autosensitization to skin are dependent upon the presence of micro-organisms. For instance, in those cases where a haematoma beneath intact skin is followed by a generalised skin reaction it is conceivable that blood-borne organisms are carried to the area, but improbable that any such organisms play any significant part.

DR J. T. INGRAM (Leeds). The granulomatous and purpuric reactions associated with allergic vasculitis may be differently influenced by steroid hormones. Do steroids play any part in the experimental work that is being done?

DR P. G. H. GELL (Birmingham). It is now, I think, generally accepted that cortisone diminishes the infiltration of cells in tuberculin reactions in the rabbit. It has of course no effect on the union of antigen and serum antibody and the effects of this union in the tissues.

DR S. T. ANNING (Leeds). I suppose that Dr Bruce tried the effect of the cream by itself and also he was, I think, comparing the effects of purified protein derivative with tuberculin. I just wondered whether the effects were the same.

DR R. A. BRUCE (Cambridge). We of course used control puncture tests on all patients and recorded no reactions at all with the plain cream base. We used purified protein derivative from tuberculin and the reactions elicited with this and O.T. are similar. We were not comparing the reactions of PPD with O.T., but endeavouring to pick out low degrees of sensitivity using purified protein derivative, which is the potent fraction of O.T.

DR T. WARD (Scunthorpe). I would like to ask Dr Bruce if he knows precisely how long this material does remain active under the skin. I wondered if this might be used to follow up people who had not had their primary infection.

BRUCE. The cream can remain inert in the skin and produces a reaction when tuberculin allergy has been established. Six weeks is the longest

observation period, so far but I imagine we might get results if we left it in for six months, a point which we hope to be able to elucidate. The reaction has been seen to persist for as long as six months and fades gradually The persistence of the reaction only causes a slight inconvenience. It would be extremely useful to use the depot cream as an indicator of primary tuberculous infections. We hope to be able to put this to the test, by implanting the cream into children at school and school leavers, inspecting their arms from time to time, and awaiting the development of tuberculin allergy

GELL. *I might add that it has been found that tuberculin tagged with radio-iodine does not appear to be more efficiently localized in the hyper sensitive than in the normal animal. Though it is possible that the tracer iodine was not bound to the active antigen in the tuberculin, the observation is nevertheless puzzling*

DR N HJORTH (Copenhagen). Lupus vulgaris may arise after B.C.G vaccination either after revaccination or in persons with a previous tuberculous infection as demonstrated by a calcified primary complex. In such cases accelerated reactions occur as a consequence of vaccination of persons showing false negative reactions to the tuberculin tests (Horwitz, 1955)

One of the reasons for the false negative Mantoux tests may have been a previously unsuspected instability of the Danish and several other PPD tuberculins. Recently it was found that partial emptying of a tuberculin vial resulted in a rapid decline of the activity of the remainder of the solution. This loss of potency sometimes resulting in a solution of 20 per cent of the original strength, may occur within one day (Magnus, Guld, Waaler & Magnusson, 1956). PPD tuberculin containing 0·005 per cent of a non-ionic emulsifier Tween 80 as stabilizing additive has been found to be stable for at least three months. The solutions containing Tween 80 show a considerably higher activity than similar solutions without this additive.

In the present paper tuberculin in an emulsifying cream was shown to give a higher incidence of positive reactions than a tuberculin solution. I wonder whether that might partly be due to different stability and actual strength of the two tuberculin preparations.

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NFLAMMATION

THE MECHANISM OF INFLAMMATION

By W G SPECTOR

Inflammation is the reaction of living tissues to injury Its macroscopic manifestations have been recognized since ancient times and its microscopical features were described a hundred years ago. In spite of this, our knowledge of the pathogenesis of the inflammatory reaction, of the mechanism of inflammation, remains rudimentary

It is the purpose of this communication to discuss the problem of the pathogenesis of the inflammatory reaction in the light of the hypothesis that there exists in the tissues an endogenous mechanism, normally dormant but capable of activation by injury that is responsible for the phenomena of inflammation.

There are sound reasons for suspecting that such a mechanism exists. First, there is the obvious fact that tissue injury of the most diverse type, from bacterial invasion to frostbite produces similar inflammatory changes. Secondly may be cited the similar pattern of inflammation in widely different species. Thirdly there is the fact that whatever the injurious stimulus, inflammation tends to proceed in an orderly sequence of events, suggesting that each phenomenon paves the way for the appearance of the next. Fourthly it may be said that in spite of this often inexorable march of events, the stimulus which initiates inflammation is often extremely transient. Fifthly there is the fact that many types of injury for example, burns, although leading to the full sequence of inflammatory changes add nothing to the tissues which might act as a material stimulus for prolonged local reactions. Finally it has been observed that the inflammatory response may not begin until some considerable time has elapsed after the application of the injurious stimulus that in other words, there is a latent period between injury and the onset of inflammation. Indeed, in some cases, inflammation may not commence until the injurious agent shows signs of being removed or destroyed by the body

It may be worth while at this point to give one or two examples of such a latent period in the onset of inflammatory changes. Before doing so, however I would like to state which inflammatory changes I propose to consider in this talk. I must say at once that I will confine myself to the changes of acute inflammation only Fascinating as chronic inflammation and the healing process are, time does not allow us to consider them. The particular phenomena of acute inflammation that I wish to discuss are

increased capillary permeability to protein, and the emigration of leucocytes. I think that most pathologists would agree that these are the cardinal features of acute inflammation.

With regard now to the latent period between injury and reaction, I would like to mention the work of Burke & Miles on bacterial inflammation. These investigators found that following cutaneous inoculation with a variety of pathogenic organisms, including *Staph. aureus*, *Strep. pyogenes* and *B. coli*, the major increase in capillary permeability did not commence until about 90 min. after the injection and was not maximal until 3–4 hr after the injection. In spite of this, previous results had shown that most of the invading organisms were killed by the body's defences in the first hour after their inoculation. It seems unlikely, therefore, that the latent period is due to the fact that it is necessary for the organisms to multiply before they can exert an effect on capillary permeability.

Inflammation of the pleura can be produced by injection of turpentine into the pleural space of rats. In this condition I found that increased capillary permeability in the pleura, as measured by the passage of plasma albumen labelled with radioactive iodine, was not maximal until 4–6 hr after the injection of irritant.

Again, in the inflammation which follows thermal injury, Sevitt (1957) found that after burns of moderate severity, increased capillary permeability in guinea pig skin, as measured by accumulation of oedema, began its most significant development 4 hr after injury and did not become maximal until 8 hr after injury. Spector & Willoughby (1958) have obtained essentially similar results in the rat.

All these results relate to increased capillary permeability. There is, however, a delay between injury and emigration of leucocytes from the vessels which is even more striking. Thus, in the bacterial invasions produced by Burke & Miles, the presence of leucocytes in the tissues was scarcely demonstrable until 1 hr had elapsed after the inoculation. A similar or even larger delay occurs in both thermal injury and chemical injury produced by turpentine.

Thus in many different types of acute inflammation a latent period exists between injury and the onset of the cardinal features of the inflammatory reaction. In inflammation following radiation injury or antigen-antibody reactions, this delay may be even more striking, but I have refrained from stressing these conditions because of their complexity. In my opinion, the most likely explanation of this latent period is that the tissue changes of acute inflammation are due at least in part to the activation of endogenous intermediary mechanisms and that a certain amount of time is needed for injury to achieve this activation.

I would now like to consider the possible forms that such an endogenous mechanism might take. It seems to me that there are two broad possibilities. First, the mechanism might reside wholly in the capillary wall, consisting perhaps of a series of enzymes and substrates subject to interference by a wide variety of injurious stimuli. However we would have to postulate that such interference always leads to similar results, namely the phenomena of acute inflammation.

The second possibility is that injury leads to the liberation or activation of endogenous chemical substances (or mediators or local hormones) which are then responsible for the changes of acute inflammation. There is little evidence of any kind, concerning the first of these two hypotheses and most of such evidence as is available is in favour of the second.

Thus the length of the latent period which often precedes the onset of acute inflammatory phenomena is, if anything, against the theory that intermediary reactions occur within the vessel wall itself Secondly there exists an important group of chemical compounds, some of known and some of unknown structure, which are capable of reproducing, in low concentration, one or more of the phenomena of acute inflammation Thirdly such compounds have been demonstrated in the vicinity of inflamed tissues at the time when they might well be expected to be exerting their effect, and to be no longer demonstrable when the inflammatory changes have subsided. Finally it has been possible to suppress the changes of acute inflammation by pre-treatment of the injured animal with measures more or less specifically antagonistic to one or other of the chemical substances believed to be responsible for these inflammatory changes.

THE PATHOGENESIS OF INCREASED CAPILLARY PERMEABILITY IN ACUTE INFLAMMATION

As I have already partly implied, there are three possible ways in which this change might take place. There might be direct physical damage to the capillaries by the injury itself. There might be activation by injury of biochemical mechanisms within the vessel wall and, finally there might be liberation of chemical mediators. Several endogenous compounds are known which increase capillary permeability in low concentrations. These are histamine, 5 hydroxytryptamine (in some species only), certain peptides and certain globulins, mucoproteins and proteases. I hope that the next speaker will say something about the action and activation of some of these substances. My purpose is to present such evidence as is available for or against their participation in the inflammatory reaction.

Histamine has been shown to be released from tissues of many species

described in serum, which was able to suppress the activity of the permeability increasing globulin.

Now the earlier exudates which you will recall possessed the ability to increase capillary permeability contained significantly less of this inhibitor substance than did the inactive, later exudates. It was in fact possible to suppress the action on capillary permeability of the permeability increasing fraction from the earlier exudates by combining it with the inhibitor fraction of the later exudates. Similarly the permeability increasing fraction of the resolving exudate did in fact increase capillary permeability when re combined with the inhibitor fraction of the earlier exudate. These results indicate that the ability of the earlier exudate to increase capillary permeability was partly due to a reduction in the amount of inhibitor available. This suggests, in turn, that as a result of injury by turpentine the inhibitor which normally ensures the inactivity of the permeability increasing globulin in serum is weakened and that the globulin is thus enabled to increase capillary permeability in the affected area. This is not the whole story however because the permeability increasing globulin exists normally as an inert precursor and unless it is activated, for example, by blood clotting or contact with glass, even total destruction of inhibitor substance will not allow it to increase capillary permeability We must postulate, therefore, that tissue injury both activates the precursor of this globulin and also weakens the action of its specific inhibitor

Before leaving this topic I would like to say that it is quite possible that these globulins do not themselves alter capillary permeability but that they might act as substrates or enzymes for the formation of active peptides. I think also that these experiments I have just described illustrate the danger of drawing conclusions from studies of inflammatory exudates without regard to the age of the exudate Inflammation is a dynamic process and it seems that in many types of acute inflammation activation of endogenous mechanisms occurs in the first few hours. Attempts to identify such mechanisms several days after the initial injury may therefore be of little value.

Dermatology I believe is often one of the more empirical branches of medical practice. I think you will agree with me therefore when I say that if a particular endogenous substance is responsible for certain inflammatory changes, the inhibition of that substance should prevent the inflammatory change for which it is believed to be responsible. *In other words, the proof of the pudding is in the eating*

I want now to describe some experiments in which the increased capillary permeability which normally followed two different types of injury was suppressed by various forms of pre-medication.

Let us take first acute pleurisy produced in rats by the intrapleural injection of turpentine. You will recall that there is some evidence that increased capillary permeability in this condition, which leads of course to the formation of the typical protein rich exudate, is *initiated* by an immediate but transient release of histamine. If this theory is correct, administration of anti-histamine drugs should have some effect on the course of events. In fact, small doses of mepyramine maleate (better known as Anthisan) no bigger than those used to control mild hay fever completely suppressed the initial formation of exudates when given 10 min. before the intrapleural injection of turpentine, the effect being seen best 30 min. after injury. However 2 hr after injection of turpentine, Anthisan-treated rats had as much exudate in their pleural cavities as did control rats. Repeated injections of Anthisan during these 2 hr failed to prevent the exudate accumulating.

When rats were depleted of their bodily histamine by repeated injections of chemical histamine liberators, and then given turpentine intrapleurally they behaved like the Anthisan-treated animals, with suppression of the initial phase only of increased capillary permeability.

These experiments suggest, therefore, that increased capillary permeability in this type of pleural inflammation at least, is in fact initiated by release of endogenous histamine and then maintained and sustained by some mechanism insensitive to anti-histamine measures.

Now you are aware that sodium salicylate is in some circumstances, for example in acute rheumatism, a powerful anti-inflammatory drug. Salicylate also has the property of preventing the conversion of permeability increasing globulins from their inactive to the active forms. If therefore, these globulins sustain increased capillary permeability after histamine has done its work, then exudate formation should be suppressed after administration of salicylate. If in fact salicylate is given together with Anthisan before intrapleural injection of turpentine, the formation of pleural exudate is suppressed altogether. If salicylate is given in this way but without Anthisan, it has little effect. We think that this is because salicylate has little effect on histamine released from the pleura and that this histamine is sufficient in itself to cause the formation of a sizeable exudate, even if the globulins are suppressed by salicylate. We know that the combined effect of salicylate and Anthisan is not due to cross-potentiation as, for example, in the case of aspirin and phenacetin, because depletion of bodily histamine is as effective in combination with salicylate as is the administration of Anthisan.

These results suggest, therefore, that increased capillary permeability in the rat's pleura, induced by the irritant action of turpentine, is initiated by

organisms could exert a true chemotactic effect on the leucocytes, drawing them irresistibly out of the vessels. Secondly leucocytes could leave capillaries merely as a consequence of increased permeability to large molecules. Thirdly injury could produce a change in the capillary wall such as would allow free passage of white cells through the structure and quite separate from increased permeability to proteins.

Chemotaxis is a positive directional response by cells to a chemical substance in their vicinity The behaviour of amoebae is probably the example best known to us. In the case of leucocytes, chemotaxis can only be proved to exist *in vitro* *In vitro* leucocytes are undoubtedly attracted by chemotaxis towards certain micro-organisms, notably *Staphylococcus aureus* and it seems very likely that the large collections of polymorphs which occur in inflammation due to pyogenic bacteria are the result of chemotaxis.

In other types of inflammation, however the role of chemotaxis is less certain, for with rigorous methods of study sterile extracts of damaged tissue have been found to exert no significant chemotactic effect on leucocytes. On the one hand it has been reported that a variety of polysaccharides derived from the tissues are in fact chemotactic to polymorpholeucocytes *in vitro* When some of these possibly chemotactic polysaccharides, for example starch are injected into the skin, the amount of leucocyte emigration they cause is negligible. On the other hand, there are some polysaccharides, particularly the acid polysaccharides dextran sulphate, chondroitin sulphate and carageenin, which is obtained from seaweed, which do cause leucocytes to leave blood vessels when they are injected in the skin of living animals.

The situation with regard to polysaccharides and leucocyte emigration can be summed up in this way Some of these substances cause leucocyte emigration in the living animal, others may be chemotactic to leucocytes *in vitro* i.e. in a test tube. No parallel study of these two properties has been carried out so we cannot yet say whether the leucocyte emigration produced by some polysaccharides is due to chemotaxis or to an effect on the capillary wall.

With regard to the possibility that leucocytes leave vessels purely as a consequence of increased capillary permeability to large molecules, the evidence is largely against such a theory It is true that all substances which increase capillary permeability also, in high concentration, induce some leucocyte emigration, but this effect is generally feeble and seems inadequate to account for the large numbers of leucocytes found in the tissues during many types of sterile inflammation. In addition, it is commonly observed that the passage of large numbers of leucocytes out of

the vessels occurs at a later stage of inflammation than that at which increased capillary permeability to protein is at its peak.

You will now want to know whether there is any evidence that endogenous mechanisms are actually responsible for leucocyte emigration in inflammation. We have already mentioned that some of the permeability increasing substances, for example histamine and globulins, demonstrated in inflamed tissues also cause leucocyte emigration. We have also said however that they exert this effect only in high concentrations and even then to a very modest degree.

Now if the hormone oestrogen is administered to animals, the uterus develops all the features of acute inflammation including massive leucocytic infiltration. Indeed, such a leucocytic infiltration is characteristic of certain stages of the oestrus cycle in mammals in general, including man. We have found that extracts of oestrogen-treated uterus from mice will cause massive leucocyte emigration on injection into the skin of living animals. This effect on leucocytes is not due to the oestrogen itself and is unrelated to any action on capillary permeability to protein. The chemical properties of the active principle suggest that it may well be a polysaccharide of the type described earlier It seems likely that this highly active substance is responsible for the leucocyte emigration that occurs in the uterus after administration of oestrogen, and during the oestrus cycle. It may well be possible to demonstrate a similar mechanism in various forms of true inflammation. Once again, however we do not know whether it works by chemotaxis acting on the leucocytes or by virtue of an action on the capillary wall which allows the white cells passage.

If endogenous intermediary mechanisms cause leucocyte emigration in inflammation it should be possible to suppress this change by previous administration of drugs which inhibit such mechanisms. In fact, if salicylate is given to rats prior to intrapleural injection of turpentine, the massive infiltration of the pleura with leucocytes which otherwise occurs is completely suppressed. If salicylate is given alone without the anti-histaminic Anthisan, leucocyte emigration is suppressed but increased capillary permeability consequent on release of histamine still occurs. In this way it is possible to dissociate the two phenomena. The pleura becomes coated with a thick layer of fibrin but is devoid of leucocytes. Salicylate also suppresses leucocyte emigration when given prior to thermal injury It has also been reported to have a similar effect on the cornea following mechanical injury Thus, although we have seen that the evidence suggests different intermediary mechanisms for increased capillary permeability to protein and leucocyte emigration, salicylate inhibits both processes.

I might leave the subject of leucocyte migration with a general statement

that polysaccharides may be involved with its development. However since this is not too solemn an occasion and since I think you would prefer me to be constructive, I prefer to put forward at least one hypothesis. I think it likely that shortly after injury the capillary wall, in addition to becoming permeable to plasma protein also becomes sticky so that leucocytes in the circulation come to adhere to the surface of the vascular endothelium. We do not know the nature of this change in the surface of the endothelium but it may be connected with alterations in surface charge. I think it possible that the next step is the liberation from these adherent, immobilized leucocytes of a polysaccharide which, either by chemotaxis or by an action on the capillary wall, leads to emigration of leucocytes on a large scale. This scheme is not entirely fanciful for we know that this very sequence of adhesion and liberation of polysaccharide from polymorphs is responsible for the pyrexia which follows injection of bacterial endotoxin.

Endothelial stickiness is very closely related to increased capillary permeability to protein. As a result, I believe that the hypothesis I have just outlined explains why salicylate suppresses both increased capillary permeability and leucocyte emigration, and also helps to explain why leucocyte migration always follows but never precedes increased capillary permeability

It is now time to conclude this talk. I would like to say that it may seem to some of you both naïve and unnecessary to postulate an all-embracing endogenous mechanism for inflammation. You may feel that external forces, especially bacteria, are quite capable of doing the job themselves without the help of intermediary mechanisms. I hope that some of the evidence I have presented in this lecture will lead you to consider that endogenous mechanisms might after all have some importance. There is no doubt whatsoever that wherever one looks in the reaction of living tissues to injury one finds that such mechanisms exist. What could be more natural than that the fever associated with bacterial invasion of the body should be due to a direct action of the bacterial toxins? But it has now been proved beyond reasonable doubt, that such bacterial toxins serve merely to release an endogenous pyrogen from leucocytes. It is thus leucocytic pyrogen alone not the bacterial toxins, which act on the thermo-regulatory centre of the brain to cause fever

It is results of this nature that encourage us to continue our experiments until such time as the pathogenesis of inflammation ceases to be a subject for hypothesis.

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ENDOGENOUS SUBSTANCES CAPABLE OF PRODUCING SOME FEATURES OF THE ACUTE INFLAMMATORY REACTION

By M SCHACHTER

The substances whose properties are briefly reviewed here are histamine, 5-hydroxytryptamine, and a group of polypeptides (kallidin, bradykinin, etc.) derived from plasma or serum, and called plasma kinins. All these substances are vaso-active and produce various features of Lewis's triple response in skin. They are highly active pharmacologically but are normally held in the organism in an inactive form. All are, however readily releasable, and the amount available is so great as to be capable of producing severe or fatal reactions should this occur A definite physiological role cannot as yet be ascribed to any of them.

HISTAMINE

Histamine (β-imidazolethylamine) is widely distributed in nature. Many animal venoms such as those of snakes, wasps and bees contain it in very high concentrations (Schachter & Tham, 1954) the hairs of the nettle plant are also a rich source (Emmelin & Feldberg 1947). It is present in variable amounts in almost all tissues of man and other mammals (Feldberg, 1956), and its pharmacological actions are multiple and diverse. It dilates (or constricts) the arterioles, increases capillary permeability contracts most smooth muscles, and causes secretion of many gland cells it stimulates sensory nerve endings in human skin producing the flare reaction, pain and itch (Lewis, 1927 Dale, 1950 Craver 1950 Keele, 1957).

Tissue histamine is probably almost entirely derived by enzymatic decarboxylation of the amino acid histidine (Schayer 1956*a*, *b*). However although histidine decarboxylase is present in many tissues of rodents, there is no convincing evidence of the presence of such an enzyme in the tissues of dog or man (Werle & Hertzer 1938 Waton, 1956) It is possible, therefore, that there is no true endogenous formation of histamine in these species. Their histamine is presumably all derived from the intestinal contents where it is formed from histidine by histidine decarboxylase of bacterial origin (Ackermann, 1910 Gale, 1953). In rodents also, despite the fact that many tissues contain the decarboxylating enzyme, some hist amine is probably derived from bacterial decarboxylation of histidine

since inhibition of the intestinal bacterial flora in rats by orally administered antibiotics greatly reduced the urinary excretion of histamine (Wilson, 1954).

In man and most other mammals histamine is located in the tissue mast cell or circulating basophil (Graham *et al* 1955 Valentine, Laurence, Pearce & Beck, 1955) Thus, in most tissues, there is a correlation between histamine concentration and mast cell content and disruption of mast cells is accompanied by a release of histamine also, pathological tissues rich in mast cells such as urticaria pigmentosa lesions in man, or mastocytomas in the dog contain very high concentrations of this amine (West, 1956). Analysis of tissue homogenates indicates that histamine is held in the mast cells within definite granules, namely in the large or mitochondrial particle fraction (MacIntosh, 1956 Fulton, Maynard, Riley & West, 1957). Heparin is also contained in the mast cell granules but it is not clear whether it exists in the same granule as the histamine (Koksal, 1953 MacIntosh, 1956). Despite the definite relationship between the mast cell and histamine, there is good evidence that in some tissues, for example, stomach and intestine, it is by no means confined to mast cells (Feldberg 1956).

To the physiologist and experimental pathologist perhaps the most interesting property of histamine is its releasability from the cells which contain it. The fact that it is released from tissues by snake venoms, trypsin, bacterial toxins, and during anaphylaxis, has been known for some time (Feldberg, 1954), but it is only fairly recently that it has been found to be released by a large group of relatively simple organic bases such as the aliphatic mono- and diamines, diamidines diguanidines and diisothioureas. Surface active compounds such as lysolecithin, bile salts and saponin are also effective under certain conditions (McIntire, 1956 Paton, 1957).

The most potent histamine releasing substance known is compound 48/80, a condensation product of *p*-methoxyphenylethylmethylamine and formaldehyde Unlike snake or other venoms which cause gross tissue damage and only incidentally release histamine, the essential pharmacological activity of 48/80 is due to its ability to release histamine. There is no gross tissue destruction following effective doses of 48/80 microscopically there is evidence of degranulation or disruption of the mast cells which have released their histamine (Paton, 1957)

Injected intradermally compound 48/80 produces a typical flare and wheal. The subcutaneous or intravenous injection of this substance (or of other specific histamine liberators) produces a characteristic syndrome of erythema, pruritus and oedema. The oedema is most marked in the face and the eyelids lips and forehead are particularly involved. This is identical with clinical angio-oedema and it occurs in laboratory animals and in man

(Paton & Schachter 1951 Norlander 1957 Lecomte, 1957) It does not occur following the administration of histamine itself In man and other mammals (excepting the rat) it is probably due entirely to the release of histamine from mast cells in the skin. In the rat, however 5-hydroxytrypt amine, which in this species is also present in the mast cells, seems to contribute to the reaction (Parratt & West, 1958).

A striking feature of the histamine release syndrome is the temporary refractory state of the animal to compound 48/80 (or other specific hist amine liberator) after one or more injections. Immediately after a severe reaction, and for some time thereafter a dog shows no response whatever to repeated administration of the drug A response may still be elicited, however by increasing the dose, or by allowing an interval of several days or more between injections (Paton & Schachter 1951). The same phenomenon has been observed in man (Lecomte, 1956). The refractory state is almost certainly due to the reduction or depletion of histamine in the skin, *and the return of sensitivity to the restoration of histamine-containing* mast cells in the tissue.

In addition to the histamine liberators already referred to, a number of drugs in clinical use have the ability to release histamine from perfused skin and other preparations. Thus, curare, morphine, pethidine, atropine, tolazoline and others, share this property although the concentrations required to release histamine are considerably greater than those required therapeutically Also, unlike 48/80 they do not produce the angio-oedema reaction in animals, presumably because their major pharmacological effects are not due to histamine release. Occasionally however they do produce angio-oedema reactions in man even in therapeutic doses. Evidence of antibodies in many drug reactions of this type is not usually demonstrable (Soderman, 1950), and the possibility should be considered that in some of these instances the reaction is not one of allergic sensitization, but evoked in some way by the primary histamine releasing property of the drug.

There is as yet no single satisfactory explanation of how histamine is held in the cell nor of how it is released. A number of theories have been postulated (*a*) That intracellular histamine is linked by a peptide or other linkage to a protein, and that activation of a proteolytic system is the basic mechanism in the release of histamine (Rocha e Silva, 1938 Ungar 1956). (*b*) That histamine is combined in the cell with heparin or some other tissue acid and that release, at least with the basic liberators, occurs by an ion exchange process (MacIntosh & Paton, 1949). (*c*) That chemical histamine releasers, or antigen-antibody reactions in anaphylaxis, activate a lecithinase on the mast cell surface, which by virtue of its own action on the membrane

phospholipid and by the lytic action of the lysolecithin produced, disrupts the granule membrane and releases histamine (Uvnäs, 1958).

None of these theories accounts satisfactorily for all the observations. The significant facts are, that histamine, in the mast cell at least, is loosely held in a mitochondrial like particle in a readily diffusible form. It is readily released from suspensions of these particles by freezing, hypotonic media, surface active agents, and by many organic bases. It is likely therefore, that histamine may be released by different mechanisms which are able to produce increased permeability or rupture of the granule membrane enclosing it in the cell.

5-HYDROXYTRYPTAMINE

Like histamine, 5-hydroxytryptamine (5HT) is widely distributed in nature. It is present in high concentrations in the venom of the toad, wasp and scorpion, and also partially accounts for the stinging properties of the nettle and cowhage plants (Collier 1958). The highest concentrations in mammalian tissues exist in blood, intestine and brain—particularly in the hypothalamus (Crawford, 1958). The 5HT in blood is located in the platelet (Humphrey & Jaques, 1954. Zucker & Borelli, 1955), except in the rat and mouse where it occurs together with histamine in the mast cell (Benditt, Wong, Arase & Roeper 1955. Parratt & West, 1956), in the gut it is in the argentaffin cell (Erspamer 1954. Lembeck, 1954. Barter & Everson-Pearse, 1955) but in brain tissue its location is not yet established. The available evidence indicates that 5HT, like histamine, is stored in specific granules of the cytoplasm (Blaschko, 1956).

5-Hydroxytryptamine in tissues is derived from the amino acid tryptophan, which is converted to 5-hydroxytryptophan (5HTP) by hydroxylation and then to 5HT by decarboxylation. Pyridoxal phosphate (vitamin B_6) is a co-enzyme required for effective conversion of 5HTP to 5HT (Buxton & Sinclair 1956) by 5HTP decarboxylase. The latter enzyme is present in many tissues, particularly those of liver, intestine and kidney but not in platelets (Gaddum & Giarman 1956). Platelets, therefore, do not make their 5HT but acquire it by their ability to absorb it from solution in plasma (Brodie 1958). The hydroxylation of tryptophan to 5HTP has been demonstrated in the venom gland of the toad (Udenfriend, 1958), and although the reaction is considered very probable in man and other mammals, the evidence is as yet indirect (Dalgliesh & Dutton, 1957). The final metabolic conversion of 5HT in man is to 5-hydroxyindoleacetic acid (5HIAA) which appears in the urine and its excretion is greatly increased in patients with argentaffin tumours. Pellagra-like symptoms occasionally

seen in patients with argentaffinoma (Dalgliesh & Dutton, 1957) may possibly be due to a secondary nicotinic acid deficiency since dietary tryptophan is a precursor of nicotinic acid, and argentaffin tumours parasitize tryptophan and divert it from the normal metabolic pathways. In these patients 5HIAA formation becomes a major rather than a minor pathway of tryptophan metabolism (Udenfriend, 1958).

Like histamine, 5HT is effective in causing various reactions in skin. In man, it produces pain and flare on intradermal injection or on application to a blister base (Keele, 1957 Herxheimer & Schachter to be published). Though it effectively increases capillary permeability in rat skin (Parratt & West, 1958), it has no such effect in man (Herxheimer & Schachter to be published). Following its slow intravenous injection (Schneckloth, Page, del Greco & Corcoran, 1957) or its release from argentaffin tumours (Biörck, Axén & Thorson, 1952 Thorson, Biörck, Björkman & Waldenström, 1954), 5HT produces intense flushing characterized by a patchy erythema and cyanosis. The Rauwolfia alkaloid, reserpine, releases 5HT from the different cells containing it, but the release is very slow compared with the explosive release of histamine by histamine liberators. The maximum rate of 5HT release from platelets *in vitro* or *in vivo* is approximately 50 per cent in 4 hr (Brodie, 1958), and there is ordinarily no acute syndrome associated with the slow release of 5HT following reserpine administration. In patients with argentaffinoma, however reserpine produces (or intensifies) the characteristic flushing reaction, a fall in blood pressure, and diarrhoea (Smith *et al* 1957), because of the large depot of 5HT in the tumour and possibly because tumour 5HT is more readily released.

5-Hydroxytryptamine is released from platelets during the anaphylactic reaction (Humphrey & Jaques, 1954 Waalkes, Weissbach, Bozicevich & Udenfriend, 1957). It is very unlikely however that this plays a significant role in acute anaphylactic shock, but it is possible that it may contribute to some specific feature of this reaction or of other allergic phenomena.

Inflammatory cardiac lesions involving the pulmonary and tricuspid valves are frequently found in patients with argentaffin tumours (Eyler Tesluk & Drake, 1956). The possibility therefore arises that these right sided heart lesions have a humoral pathogenesis, due to the high concentration of 5HT (or other tumour metabolite) reaching the heart from the primary intestinal and metastatic tumour tissue. The absence of left-sided heart lesions would be explained by the dilution of the tumour metabolic products in the lungs or by inactivation of 5HT by the large amounts of monoamine oxidase present in this organ (Blaschko 1956). A hypothesis of this nature is supported by recent reports of two cases of argentaffinoma with mitral stenosis in addition to the usual right-sided valve lesions

released into inflammatory exudates irrespective of whether they are primarily involved in the genesis of the reaction or not. Knowledge of the properties of these and other similar endogenous systems is therefore essential to the analysis of the processes producing tissue reactions after different forms of injury

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seen in patients with argentaffinoma (Dalgliesh & Dutton, 1957) may possibly be due to a secondary nicotinic acid deficiency since dietary tryptophan is a precursor of nicotinic acid, and argentaffin tumours parasitize tryptophan and divert it from the normal metabolic pathways. In these patients 5HIAA formation becomes a major rather than a minor pathway of tryptophan metabolism (Udenfriend, 1958)

Like histamine, 5HT is effective in causing various reactions in skin. In man, it produces pain and flare on intradermal injection or on application to a blister base (Keele, 1957 Herxheimer & Schachter to be published). Though it effectively increases capillary permeability in rat skin (Parratt & West, 1958) it has no such effect in man (Herxheimer & Schachter to be published) Following its slow intravenous injection (Schneckloth, Page, del Greco & Corcoran, 1957) or its release from argentaffin tumours (Biörck, Axén & Thorson, 1952 Thorson, Biörck, Björkman & Waldenström, 1954), 5HT produces intense flushing characterized by a patchy erythema and cyanosis. The Rauwolfia alkaloid, reserpine, releases 5HT from the different cells containing it, but the release is very slow compared with the explosive release of histamine by histamine liberators. The maximum rate of 5HT release from platelets *in vitro* or *in vivo* is approximately 50 per cent in 4 hr (Brodie, 1958), and there is ordinarily no acute syndrome associated with the slow release of 5HT following reserpine administration. In patients with argentaffinoma, however reserpine produces (or intensifies) the characteristic flushing reaction, a fall in blood pressure, and diarrhoea (Smith *et al.* 1957) because of the large depot of 5HT in the tumour and possibly because tumour 5HT is more readily released.

5-Hydroxytryptamine is released from platelets during the anaphylactic reaction (Humphrey & Jaques, 1954 Waalkes, Weissbach Bozicevich & Udenfriend, 1957) It is very unlikely however that this plays a significant role in acute anaphylactic shock, but it is possible that it may contribute to some specific feature of this reaction or of other allergic phenomena.

Inflammatory cardiac lesions involving the pulmonary and tricuspid valves are frequently found in patients with argentaffin tumours (Eyler Tesluk & Drake, 1956). The possibility therefore arises that these right sided heart lesions have a humoral pathogenesis, due to the high concentration of 5HT (or other tumour metabolite) reaching the heart from the primary intestinal and metastatic tumour tissue. The absence of left-sided heart lesions would be explained by the dilution of the tumour metabolic products in the lungs or by inactivation of 5HT by the large amounts of monoamino oxidase present in this organ (Blaschko 1956). A hypothesis of this nature is supported by recent reports of two cases of argentaffinoma with mitral stenosis in addition to the usual right-sided valve lesions

(McKusick, 1956 Davies, Wolfe, Mathias & Schachter to be published). At post-mortem examination both these patients were found to have a patent *foramen ovale* which enabled the venous blood to partially short circuit the pulmonary circulation. As yet, however there is no experimental evidence that 5HT produces chronic inflammatory reactions in the heart valves.

The problem of how 5HT is held and released from cells is not solved. Although less is known about it than about histamine, 5HT also appears to be stored in intracellular granules (Blaschko 1956), where it is enclosed in a diffusible form, or readily dissociable from a loose chemical complex to become diffusible (Brodie 1958)

KININS (POLYPEPTIDES)

The term kinins has been given to a group of similar substances, probably polypeptides, which have many properties in common. This term was selected because of their ability to contract smooth muscle in a characteristic way producing a delayed, slow contraction. They are all potent vasodilator agents, they increase capillary permeability and cause pain when applied to an exposed blister base on human skin (Holdstock, Mathias & Schachter 1957).

The plasma kinins (kallidin bradykinin) are released from an α_2 globulin of plasma by kallikrein (Werle, 1955) or by trypsin and snake venoms (Rocha e Silva 1955) respectively The kinins released by these agents from plasma are closely similar or possibly identical (Werle, Kehl & Koebke 1950 Holdstock *et al* 1957 Mathias & Schachter 1958). Plasma or serum also releases a kinin by simple physical treatments such as contact with glass (Armstrong Jepson, Keele & Stewart, 1957) or by dilution (Schachter 1955). The suggestion has been made that the latter procedures activate kallikreinogen (the inactive plasma precursor of kallikrein) or a similar substance, which then splits off the active polypeptide from its globulin substrate in plasma (Schachter 1956). The amount of kallikreinogen activable in serum is such that the intravenous injection of kallikrein derived from 1·0 ml. of serum will produce a severe and protracted hypotension (Frey Kraut & Werle 1950). These facts indicate that there exist in the blood plasma the necessary mechanisms for releasing large amounts of pharmacologically active polypeptides (kinins). Although these mechanisms are readily activable *in vitro* there is as yet no definite knowledge of their role in physiology or pathology Substances resembling plasma kinins also appear in the blood of animals after injection of histamine releasers (Paton, 1951 Jaques & Schachter 1954), but in these instances

the substance has not yet been extracted from plasma and more thoroughly compared with the known kinins.

Kallikrein, the agent in plasma which releases a kinin (kallidin) from its plasma substrate (kallidinogen), is a non-dialysable, heat labile substance, with the properties of a protein. It is present in an active form in saliva and urine and in an inactive form in pancreas and blood (Werle, 1955). Kallikreinogen, its inactive precursor can be activated by careful acidification, by proteolytic enzymes, or by addition of acetone—procedures which presumably dissociate an inactivating molecule from kallikrein The different kallikreins show slight differences from one another particularly in their susceptibility to various inhibitors of trypsin (Werle, 1955) The main pharmacological effect of the kallikreins is a marked vasodilatation which is probably indirect, that is, due to the release of the plasma kinin, kallidin. Kallikrein itself, like the kinins, is also effective in increasing capillary permeability in animals (Szakall, 1934 Rocha e Silva, 1940) and in man (Herxheimer & Schachter to be published)

In recent years various experiments have been reported which indicate the involvement of proteins or polypeptides in the regulation of the vascular bed in normal or inflamed tissues. Thus, a bradykinin releasing enzyme (Hilton & Lewis, 1955) in the salivary gland has been suggested as a regulator of the blood supply to the gland during activity exudin (Menkin, 1956), enzyme like globulin in plasma (Wilhelm, Miles & MacKay 1955 Mill, Elder Miles & Wilhelm, 1958) and a globulin system controlling capillary permeability (Spector 1957) have been described and suggested as endogenous mediators in inflammation contact factor (Margolis, 1957) is the name given to the glass activated substance in plasma which increases capillary permeability and releases a kinin from plasma. All these substances share many properties with kallikrein, and it is desirable that they should be compared and distinguished from kallikrein if they are not identical with it.

GENERAL

The present review is not meant to imply that the inflammatory reaction is always endogenously mediated nor that any of the above substances are essential to it. It is intended, however to describe the facts concerning some releasable, pharmacologically active agents in blood and other tissues, which have the properties of producing pain, oedema, erythema, and pruritus in varying degree. The facts indicate that these substances undoubtedly contribute to certain inflammatory phenomena. On the other hand, they equally indicate that these substances are very likely to be

released into inflammatory exudates irrespective of whether they are primarily involved in the genesis of the reaction or not. Knowledge of the properties of these and other similar endogenous systems is therefore essential to the analysis of the processes producing tissue reactions after different forms of injury

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DISCUSSION

Chairman DR J. H. TWISTON DAVIES (London)

DR J. MARTIN BEARE (Belfast). I would like to ask Dr Schachter to comment on the 5HT in the human mast cell. I understand that Dr West who has worked on mast cell material has been quite unable to demonstrate 5 hydroxytryptamine in the human mast cell using the same techniques that he used successfully to demonstrate histamine. There are certain systemic mast cell diseases which have signs and symptoms which can be explained, I think, if 5HT was present (Beare *et al.* 1958). The gastro-enteritis and the flushing might be due to histamine, but might also be due to 5HT. They apparently only occur constantly with systemic mast cell disease and it would be nice from our point of view as dermatologists if the human mast cell did actually contain 5HT.

DR M. SCHACHTER (London). The available evidence indicates that the human mast cell contains no 5 hydroxytryptamine. However like the mast cells of most other animals it does contain histamine. Only rat and mouse mast cells have been shown to contain 5 hydroxytryptamine as well as histamine (Benditt, Wong, Arase & Roeper 1955; Parratt & West, 1956). Also, human urticaria pigmentosa lesions (and canine mastocytomas) are rich in histamine but contain no 5-hydroxytryptamine (West, 1956). All the facts are therefore consistent with the possibility that the flushing and diarrhoea seen in patients with urticaria pigmentosa are due to the release of histamine from the mast cells present in these lesions.

DR I. B. SNEDDON (Sheffield). I would like to ask Dr Schachter whether there is any easy practical method of estimating the amount of histamine released in a case of urticaria.

Is there any degradation product similar to the one produced by 5HT which can be found in the urine?

SCHACHTER. The metabolic fate of released histamine is not adequately known in man at present, and there is no simple and practical method for the demonstration of a histamine derivative in urine as is the case with 5-hydroxyindoleacetic acid when 5 hydroxytryptamine is released in excess. It is possible to measure the output of histamine in urine but this involves concentrating the histamine with columns of ion-exchange resins and then measuring the histamine by bio-assay. This, of course, has been done but it is not a very practical procedure.

Measurement of plasma histamine concentrations would not necessarily be significant if negative results were obtained. For example it is possible

to inject histamine in amounts sufficient to produce effects such as marked gastric secretion, yet there is no detectable rise in the plasma histamine concentration. Of course, in severe anaphylaxis, or when histamine is deliberately released experimentally then increased concentrations of plasma histamine are demonstrable. However it is certainly possible that urticarial and other clinical disturbances may well be due to the release of histamine which cannot be demonstrated in this way

DR S P HALL-SMITH (Hove) Am I right in assuming from what has been said that 5HT and polypeptides are as important as histamine in the production of urticaria and if so would that be the reason why anti histamines are often disappointing in treatment? Cortisone is sometimes effective in the treatment of urticaria when the anti-histamines fail I wonder if any work has been done on the level at which cortisone works in the inhibition of 5HT and polypeptides.

DR C. H WHITTLE (Cambridge). Is it legitimate to conclude that when anti histamines do not work, where we might reasonably expect them to—for instance in the urticarias and in some other conditions—that the hist amine is in fact not playing an important part in that reaction?

SCHACHTER. I have very little experience with clinical urticaria but from my observations of the actions of 5-hydroxytryptamine, I would very much doubt that it is likely to be a significant factor in this disturbance, since it does not increase capillary permeability in man (Herxheimer & Schachter to be published). The polypeptides which have been discussed are effective whealing agents and it is possible that they may be of significance.

Cortisone has no significant antagonistic action on the pharmacological actions of histamine or 5-hydroxytryptamine and any value it may possess therapeutically cannot be related to such an action. It has been claimed that cortisone interferes with the resynthesis of histamine once it has been released (Halpern, 1956).

The third question is whether the ineffectiveness of the anti histamines may be taken as evidence that histamine is not playing an important part in the reaction. One can readily antagonize most actions of injected hist amine with anti histamine drugs but it is much more difficult to block the cutaneous effects of endogenous histamine—for example, the histamine released from the mast cells in the skin following injection of a histamine liberator I do not know the explanation for this difference unless the histamine is released from mast cells in high local concentrations which cannot be simulated by intravenous or subcutaneous histamine without producing concentrations elsewhere which would prove fatal. Although the urticaria, pruritus and angio-oedema reaction associated with the release of endogenous histamine in dogs can be considerably reduced by treatment

with anti histamine drugs, the syndrome is never abolished. Therefore I do not think that one can dismiss histamine as a major factor in a clinical disturbance if anti histamine treatment is only partially effective.

DR J T INGRAM (Leeds) Is there any evidence of histamine deficiency in patients who are on long term antibiotics? Ought we to direct our attentions to the bowel in our attack on urticaria?

SCHACHTER. Sterilization of the bowel by antibiotics greatly reduces the urinary excretion of histamine in some animals (Wilson, 1954). I do not think that this effect has been studied in man, but I am almost certain that the oral administration of antibiotics would here greatly reduce the excretion of histamine and possibly tissue histamine concentrations as well. Human tissues are apparently unable to convert histidine to histamine (Waton, 1956) Histamine, in man appears to be derived entirely from the intestinal contents where it is formed from dietary histidine by histidine decarboxylase of bacterial origin. It certainly would be extremely interesting if some urticarial reactions were improved by oral antibiotic therapy

INGRAM. Histamine being so essential in so many activities this deficiency might be serious.

SCHACHTER. Not necessarily since we really do not know how essential histamine is. So far its functions seem entirely detrimental!

DR G C WELLS (London). I think that Dr Spector has done a lot of important work on the polypeptides connected with inflammation. I wondered how specific or how narrow a chemical spectrum of these polypeptides he is dealing with, say in experimental burns.

DR W G SPECTOR (London). The original idea was that one might well be dealing with a very wide spectrum of peptides as a result of general proteolysis, but opinion at the moment favours the view that we may in fact be dealing with one or more specific polypeptides formed by a particular endogenous mechanism. On the other hand, the pharmacological properties we are discussing are shared by peptides from all sorts of different sources Such peptides tend to have a lot of things in common for example, they all tend to be basic and migrate correspondingly on electrophoresis. To sum up although large numbers of peptides cause hyperaemia and increased capillary permeability the probability is that in inflammation in any given species only one or two particular peptides are involved.

DR A. BIGHAM (Bradford). Am I right in thinking that the salicylates are used as histamine inhibitors in these experiments? If so, has it been established that the salicylates do not cause histamine release in animals? I believe they have been known to cause this in man.

SPECTOR. I have not used it as a histamine inhibitor but I have used it to suppress the phase of inflammation which we thought to be subsequent

to the release of histamine. The behaviour of salicylate in experiments on the rat suggested that it had little effect on the initial phase of inflammation in the pleura, indicating that it had little anti-histamine action in that particular tissue. On the other hand, in burns of the skin in the same species it may well be that salicylate does have some anti-histamine action or some ability to oppose the release of histamine.

I do not think that salicylate releases histamine in animals, though I know it is said to do so in man. Perhaps Dr Schachter has some other information.

SCHACHTER. I do not think that salicylates release histamine in animals or man as a pharmacological property as many organic bases do. It is possible that in rare instances in man it may function as a hapten, or in some other way become antigenic and so give rise to histamine release on readministration.

DR G. A. BECK (Peterborough). I would like to ask whether anti-histamine drugs all work at the same point.

SCHACHTER. Most anti-histamine drugs are synthetic analogues of histamine. The hypotheses assumes that because of their structural similarity to histamine, they occupy the same receptor site and so prevent histamine itself from being pharmacologically effective—so-called competitive inhibition. This is the principle on which the synthesis of drug antagonists, in general, is based. In the case of the anti-histamine drugs there is the discrepancy that they are essentially ineffective in blocking the gastric secretory effect of histamine. I have no idea why this is so.

DR M. FEIWEL (London). What are the time relationships of the release of histamine from mast cells? I think your graphs showed that there was a 10 min. delay although you talk of explosive violence of histamine release by histamine releasing agents. Am I right in concluding that there is that delay after injection of 48/80?

SCHACHTER. There is a delay only of seconds in the release of histamine following injection of 48/80. The graphs presented showed the amount of histamine in the venous effluent of the perfused skin over a 10 min. period. This is simply an arbitrary and convenient interval for each sample to be assayed but histamine appears in the effluent within seconds after arterial injection of 48/80 in this preparation. This is also true if 48/80 is injected intravenously in the intact animal.

DR J. H. TWISTON DAVIES. I believe there must be something very mysterious about stickiness at microscopic dimensions and I hope that Dr Spector will be able to tell us about it.

SPECTOR. It is an extremely difficult question, but it can really be answered at two levels. By stickiness we mean the ability of the vascular

endothelium to attract and hold leucocytes. Vascular endothelium in this condition becomes literally sticky not only to leucocytes but also to particles in general. If you inject indian ink particles into the circulation they adhere to the endothelium of inflamed capillaries just as do leucocytes. That is all we know except that endothelial stickiness develops in parallel with two other changes, first with increase of capillary permeability to protein, secondly with swelling of the endothelium. These changes in capillaries are associated usually with dilatation of the capillary lumen. If we go more deeply into the question of endothelial stickiness we find it is essentially a matter for the physical chemists. What may occur is some change at the surface of the endothelial cell involving an alteration in net electrical charge or in the configuration of the chemical groupings exposed on the cell surface. As a result the sticky cell may become coated with a layer of plasma protein and in the presence of calcium this layer of plasma protein may bind the surface of leucocytes to the corresponding cell surface of the endothelial cell.

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CARCINOGENESIS

CURRENT CONCEPTS OF CARCINOGENESIS

By L. FOULDS

Clinical experience and laboratory experiments combine to show that the emergence of recognizable and classifiable lesions is a relatively late event in a process of development, which extends far backwards to inconspicuous beginnings and which can be extended forwards in the laboratory without set limit, by means of transplantation in animals or tissue culture *in vitro* For some years my own interests have been directed to the middle range where clinical observation and laboratory experiment come closest together I propose to start in the middle rather than at the beginning to introduce some concepts of neoplasia which are relevant to the consideration of earlier stages. In particular I wish to present neoplasia as a dynamic process of development which often advances discontinuously through qualitatively different stages as a result of *progression* by which is meant irreversible change in one or more of the characters of neoplastic cells (Foulds, 1954). As a matter of convenience I will use for illustration the form of neoplasia with which I have been most concerned personally namely mammary neoplasia in mice.

It is widely agreed that in most of the inbred strains of mice highly prone to mammary neoplasia the clinical tumours usually develop from localized proliferations of mammary tissue called hyperplastic nodules. At post mortem examination these nodules are on the borderline of visibility to the naked eye they are well below the threshold of clinical recognition. The nodules are present often in large, and sometimes enormous, numbers but most of them either persist indolently or regress and only one or two or a few of them advance to clinical neoplasia. In some strains of mice the hyperplastic nodules are less conspicuous and most of the tumours develop not from nodules but from plaques, which though often multiple are not numerous. The plaques, being roughly ten or more times as big as nodules, are above the threshold of clinical recognition. It is believed, though difficult to prove, that some tumours in various kinds of mice develop directly from apparently normal mammary tissue without traversing the intermediate stages of nodule or plaque. These three paths of development, the one *direct* and the other two *indirect* lead to tumours of similar common place types. The intermediate lesions on the indirect paths differ in size but probably represent comparable biological stages of development. The advance to carcinoma is not a mere growth of nodule or plaque it involves

an irreversible qualitative change, or progression, which is sometimes clearly manifested as a focal histological change within the nodule or plaque (Foulds, 1956) It is noteworthy that intermediate lesions are subject to varied fates. Nodules may regress, persist indolently or undergo progression and the progression is usually focal. In general progression takes place in only a small fraction of the available or competent tissue in the intermediate lesions.

The plaques give further information about progression by reason of their accessibility to clinical examination. The plaques and some larger growths in these particular mice are notably responsive to reproductive activity of the host they grow during pregnancy and regress after parturition. The mechanism of this phenomenon has not been elucidated but it is presumably hormonal. The immediate interest is that it provides a clinically recognizable character that undergoes progression the tumours lose their responsiveness and grow progressively thereafter without regard to pregnancy and parturition. The tumours are usually multiple so that it is possible to study the growth rate and pregnancy responsiveness of several tumours in one animal at the same time. Infrequently progression in growth rate is evidenced by a sudden acceleration. Much more often *progression is from the pregnancy responsive to the pregnancy-unresponsive state.* Several general principles of tumour progression emerging from the study of mammary neoplasia in several hundred mice have proved applicable also to epidermal neoplasia in mice (Shubik, Baserga & Ritchie 1953) and to many forms of neoplasia in man (Foulds, 1954, 1958*b*).

As a rule progression occurs in only one of multiple tumours at one time. This *independent progression of tumours* is in harmony with the principle already inferred from observations of a different kind that only a fraction of the available, competent, tissue undergoes progression. It shows that progression depends on a qualitative change in one part of a tissue and not on a change in the environment to which all parts are exposed. The time and localization of progression are unpredictable It may occur in small tumours or in large ones and either early or late in their clinical course. At its earliest clinical emergence a tumour may be at any stage of progression. The abuses of the term early are becoming recognized in clinical practice a tumour assessed as early on account of its small size or short clinical duration may be at a late stage of progression towards highly malignant behaviour Progression can lead to varied end-points but an end point is not necessarily reached during the life-time of the host progression can halt for a long time, or as it seems permanently at any stage. Different identifiable characters of a tumour for example growth rate and responsiveness to pregnancy undergo progression independently

of one another This leads to a more general proposition, which is supported by varied evidence from many sources, that the structure and behaviour of tumours are determined by numerous unit characters, which, within wide limits, are independently variable, subject to highly varied associations and liable to independent progression (Foulds, 1958*a*, *b*) It is a serious fault of the orthodox terminology of neoplasia that there are no adequate terms to cover the wide range of varied combinations of growth rate, invasiveness, powers of metastases, responsiveness to hormones, histological type and other characters. This lack of a suitable nomenclature gravely hampers the study of neoplastic development as witnessed by the tiresome disputations about the dispensable term precancerous One urgent need is for a term to cover the neoplastic lesions that lack one or more of the unit characters that are needed to satisfy all the orthodox criteria for the diagnosis of benign or malignant tumour To meet this need I propose the term *imperfect neoplasia* (or *neoplasia imperfecta*), with subdivisions into imperfect carcinoma and imperfect papilloma or adenoma for the epithelial varieties.

Table 1 *Imperfect neoplasia*

Neoplasms lacking one or more of the unit characters which combine to give the cardinal features of complete benign or malignant neoplasia.

I. Imperfect carcinoma
  (1) Conditional, dependent or responsive carcinomas whose growth is regulated by known extrinsic stimuli
  (2) Invasive but non-metastasizing carcinoma, for example basal-cell carcinoma
  (3) Non-invasive, non-metastasizing carcinoma, for example carcinoma *in situ* Bowen disease
  (4) Regressing carcinoma, for example kerato-acanthoma

II. Imperfect papilloma or adenoma
  ( ) Conditional, dependent or responsive papilloma or adenoma
  ( ) Regressing papilloma or adenoma, for example various keratoses and precancerous

The imperfect carcinomas are divisible into at least four groups (Table 1). The first group includes tumours which are typical carcinomas in every respect save that their growth is conditional, responsive, or dependent, being regulated to a greater or less extent by known extrinsic stimuli. The best-known examples are the hormone-responsive cancers of the human breast and prostate the diagnosis of carcinoma is not questioned. A second group includes tumours like basal-cell epithelioma or juvenile melanoma that invade but do not metastasize. Next come the tumours that neither invade nor disseminate they include the various forms of intra epithelial carcinoma or carcinoma *in situ*, of which Bowen s disease is an example. Lastly there is a group of non progressive or regressing tumours to which kerato-acanthoma seems to belong Descending this list, the cardinal features of carcinoma are whittled away to the point where, in the

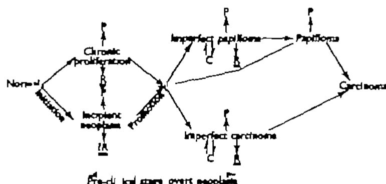
fourth group many pathologists and clinicians would refuse to use the word carcinoma and with good reason unless the term is explicitly qualified. Fairly recently it has been recognized, notably by Dr Whiteley (1957), that kerato-acanthoma closely resembles the cancroids or carcinomatoids induced by tar or carcinogenic hydrocarbons in the skin of rabbits and, less conspicuously of mice. The carcinomatoids are neoplastic but they are neither true carcinomas nor papillomas. They are best classed, as I believe, as imperfect carcinomas lacking the capacity for sustained, progressive growth. All the imperfect carcinomas have some of the characters of carcinoma, but lack one or more of the characters of true carcinoma they are all neoplastic and they are all plainly different cytologically histologically or biologically from any perfect or imperfect papilloma or adenoma. The imperfect papillomas are well exemplified by the various keratoses and precanceroses of the human skin and of rabbit or mouse skin subjected to chemical carcinogens.

Some of these imperfect neoplasias are important as potential precursors of true carcinoma. Carcinoma *in situ* of the uterine cervix, according to current opinion, is a frequent antecedent of cervix cancer which it precedes on the average by about ten years. Bowen's disease terminates in invasive cancer probably less frequently but similarly after a long time *in situ*. These imperfect carcinomas persist as such for many years and terminate in invasive carcinoma only as a result of irreversible qualitative change by progression whereby they acquire powers of invasion. Together with other imperfect carcinomas and papillomas, they are precancerous in the sense of being liable to progression to carcinoma they are not precancerous in the sense that they become carcinomas by mere extension in space and time or in the sense that progression to carcinoma is inevitable

INDUCED NEOPLASIA OF THE SKIN

The general features of induced neoplasia of the skin are similar in animals and in man and the diagram illustrates the main paths of development (Fig 1). The pre-clinical *induction* phase is long in the occupational cancers of man it extends over many years and sometimes even for many years after the last exposure to the known or presumed carcinogenic agent. The usual reactions to chemical carcinogens include acute and chronic inflammation, disturbances of pigmentation and hyperplastic lesions ranging from hyperkeratosis through various kinds of warts or papillomas to carcinoma. The lesions are commonly multiple and varied and a large proportion of them are imperfect neoplasias There are several kinds of papilloma some are conditional tumours that regress on the withdrawal of carci

nogen and others regress despite continued exposure others persist indolently or grow progressively as papillomas and others again undergo progression to carcinoma. The progression is usually a focal change in the base of a papilloma and it involves a permanent qualitative change that confers new properties on the neoplastic cells, the change not being in growth-rate alone. Most carcinomas in rabbits and mice originate by progression in papillomas or less often, in carcinomatoids, but some emerge as true carcinomas from hyperplastic or apparently normal skin. It is noteworthy that after successful removal of an early tumour or recession of all early lesions, in experimental animals and in man, new tumours may



P Persistence without qualitative change C = Conditional growth R = Regression

Fig Epidermal carcinogenesis.

continue to develop successively without further exposure to carcinogen and throughout remaining life. The skin remains permanently in a state of what I propose to call *incipient neoplasia* (or *neoplasia incipiens*).

There is sound evidence for all the paths of development shown in Fig. 1 but far more information is needed, and is obtainable, about the fate of individual lesions which have not always received sufficient attention either in dermatology or in experimental cancer research. Recent developments in experimental carcinogenesis show that the qualitative differences between individual lesions in one individual or in individuals submitted to different carcinogenic procedures cannot be ignored in the assessment of current concepts. Experimental investigations have been focused predominantly on the induction phase, sometimes with too little consideration of what is being induced and how

The study of the induction phase, conducted under different conditions in rabbits and in mice, has led to the wide adoption of a two-stage hypo-

thesis of chemical carcinogenesis, the two stages being called *initiation* and *promotion*. Peyton Rous and his colleagues working with tarred rabbits, found that when early warts regressed after withdrawal of tar the skin did not revert to its original state although it regained its normal appearance, because renewed tarring quickly elicited warts with the same specific characters and in the same places as those that had regressed moreover similar true recurrence was induced by wound healing and by some non-specific irritants, which, by themselves, had no carcinogenic action on normal skin. Rous distinguished, first, a process of *initiation* whereby normal skin is converted into a *subthreshold neoplastic state* characterized by the presence of *latent tumour cells* and, secondly a process of *promotion* which stimulated the latent tumour cells to proliferate and form visible tumours. Initiation and promotion were regarded as distinct and different processes. The chemical carcinogens exerted both initiating and promoting action, although in varied degrees, but the promoting agents were not carcinogenic in the full sense.

To account for the phenomenon, already mentioned, of continued emergence of new tumours for long periods after the last exposure to carcinogen, Rous supposes that, as well as initiating latent tumour cells, the carcinogen initiates *latent neoplastic potentialities* in cells which then advance automatically but gradually and at varied, often extremely slow rates towards the neoplastic state.

In mice the chemical carcinogens evoke papillomas like those in rabbits but more of them undergo progression to carcinoma relatively early Repeated applications of carcinogen induce at least one carcinoma in almost every mouse. As the carcinogenic stimulus is reduced, fewer warts develop and fewer become malignant. A single application of carcinogen, depending on the potency of the agent and the sensitivity of the mouse induces as a rule, within the usual period of observation, either no tumours or only a few long-delayed warts most of which remain benign. After such weak carcinogenic action, certain agents, of which croton oil is the most potent and the most used, when applied repeatedly for two or three months, evoke a large crop of papillomas most of which remain benign. Berenblum & Shubik (1947) interpreted experiments of this kind in terms of *initiation* and *promotion* as used by Rous and their example has been widely followed. Like Rous they believed that the mechanisms of initiation and promotion were entirely different and that the combined effect was not attributable to summation of comparable stimuli but to stimuli of different kinds operating consecutively

Subsequently many more promoting agents have been studied, amongst them detergents of the Tween series. Even more interestingly urethane

and some other substances have been found to act as initiating agents. The term *incomplete carcinogen* has been applied to agents like urethane and croton oil which seem capable of effecting one stage of the carcinogenic process but not all of it. It now seems that all these agents can be fully carcinogenic when adequately tested over sufficiently prolonged periods of time. This does not invalidate the general principle that initiation and promotion are different and consecutive processes, or minimize the value of the incomplete carcinogens, with their great preponderance of one or the other action, for the analysis of the two processes.

In the earlier histological investigations of epidermal carcinogenesis induced by complete carcinogens the effects of the initiation and promotion were not separable. The total effect of the carcinogens was to produce hyperplasia with delayed maturation of epithelial cells tumours developed as a rule from localized areas in the widely hyperplastic epithelium. Urethane is reported to effect no recognizable change in mouse skin when applied locally Moreover urethane and the complete carcinogens can bring about initiation in the skin when administered remotely by mouth or by parenteral injection the initiation is disclosed by the growth of papil lomas at the site of application of croton oil to the skin. So far no visible effect of the remotely administered agent on the skin has been described. Promoting action is not proportional to the hyperplasia and comparable degrees of hyperplasia evoked by other irritants have no promoting effect. The promoting action seems therefore to depend on some peculiarity of the promoting agent whether chemical or as Setala and his colleagues infer from their work with the Tween detergents, physico-chemical (Dammert, 1957). Berenblum believes that promoting agents delay cell maturation but Setala ascribes this action to the initiating agent. It is noteworthy that promotion in mice depends on continuous action of the promoting agent, prolonged until near the time of emergence of tumours.

The number and the biological characters of the tumours produced in these initiation promotion experiments depend predominantly on the initiating stimulus the promoting agent determines the location and time of emergence. When initiation is effected by an incomplete carcinogen such as urethane or by minimal applications of a complete carcinogen the tumours evoked by subsequent applications of croton oil are mostly papil lomas, many of them imperfect, that seldom undergo progression to carcinoma. Carcinoma may develop late, not necessarily from papillomas, in most experiments using borderline initiating action but promoting agents seem to have no effect on their emergence and no effect on the progression of papilloma to carcinoma. Dammert (1957) suggests that promoting action may be limited to the promotion of papillomas. Although

have probably discouraged repetition of the experiments on a large scale New methods of continuous cultivation of normal animal and human cells are now in use. The usual experience is that sooner or later the cultures deteriorate and eventually die out unless some cells undergo permanent change and establish a new race of cells that grow vigorously and in definitely Some cytologists believe that the altered cells are malignant but have not yet supplied proof (Leighton 1957). Whether the altered cells are neoplastic or not, it is clear that normal cells growing in abnormal conditions *in vitro* often undergo a permanent change, or progression, which establishes new properties including the ability to proliferate well in conditions formerly inimical to them.

VIRUS NEOPLASIA

The Rous sarcoma is the best known member of a large group of filterable sarcomas and leukaemias of the domestic fowl that are transmissible by replicating subcellular particles with the properties of viruses. The transmission differs in several respects from other carcinogenic processes (1) The transformation of normal into neoplastic cells is rapid and perhaps almost immediate. (2) The transformation is *direct* from normal to sarcoma cell with no intermediate precursor or precancerous stages. (3) The transformation is qualitatively specific. The tumours are varied in structure and behaviour Each kind of tumour has its specific filterable agent, which exactly reproduces the characters of the particular kind of tumour from which it was extracted. The transmission is a process of tumour re production rather than induction. (4) The agent is recoverable in greatly increased quantity from the tumours it evokes.

The Rous agent contains protein and ribosenucleic acid one of which, or both together must be credited with specific structure to account for the high specificity of the biological action. The agent impresses a specific type of structure and behaviour upon the cells it transforms. The imposed characters are heritable being transmitted to the progeny of the transformed cells, and the agent is recoverable from the descendant cells. There is an evident resemblance to the phenomena of *transformation* and *transduction* in bacteria where it is believed, genetic material in the form of a pentose nucleic acid is removed from one cell and transferred to another cell into whose genetic material it becomes incorporated, thereby impressing specific characters of the donor cell upon the recipient cell. Similarly it is likely that the Rous sarcoma agent, or some part of it, becomes incorporated in, and modifies, heritable material in the nucleus or cytoplasm of cells that are thus transformed into sarcoma cells (Foulds 1958*c*). The outstanding

if insufficiently recognized, importance of the fowl tumours lies in the opportunities they promise for the analysis of intra-cellular mechanisms of neoplasia.

It is not yet demonstrated that a comparable relationship between agent and neoplastic cell obtains in the more recently discovered examples of virus tumours which form a heterogeneous group Current interest is centred on the notable success of Gross, Stewart, Friend and others in producing leukaemias and some other neoplastic diseases in mice and even in hamsters by the injection of tissue extracts or cultures. The mechanism is not yet elucidated. The Bittner milk agent, sometimes described as the cause of mammary cancer in mice is not indispensable for that disease in mice and is not found in other animals. According to much current opinion, the essential action of the milk agent is to enhance and accelerate a neoplastic process that advances slowly in its absence. The action of the Shope papilloma virus of rabbits is more clearly demonstrated. This undisputed virus evokes papillomas of the skin of rabbits. Eventually these papillomas either regress or undergo progression to carcinoma. The development to carcinoma is always indirect and independent of the virus. The Shope virus induces papillomas It does not induce carcinomas or progression from papilloma to carcinoma and it is not recoverable from the carcinomas. Thus the Shope virus undoubtedly induces neoplasia but the relationship of virus to the definitive tumour cells is certainly different from that in the filterable tumours of fowls.

CONCEPTS OF CARCINOGENESIS

The concepts of carcinogen and carcinogenesis are becoming increasingly vague. The known methods of inducing neoplasia are numerous and varied (Haddow 1958). As Berenblum (1956) remarks, there is no foundation for a belief that the same mechanism operates in all of them. Currently at least three different mechanisms seem probable. The filterable agents of fowl sarcomas and leukaemias stand apart in converting normal cells directly into neoplastic cells of qualitatively specific types, the conversion probably depending upon an incorporation of specific material (protein, nucleic acid or nucleoprotein) into heritable replicating mechanisms of previously normal cells. The carcinogenic polycyclic hydrocarbons and azo compounds probably have a direct chemical action on cell constituents, variously identified, but many steps are interposed between their initial action and the definitive tumour Many carcinogens, chemical and physical, probably do not directly alter the cells destined to become neoplastic but act indirectly by bringing about a pathologically maintained proliferation of those cells.

In hormone-induced neoplasia, the essential factor seems to be long continued proliferation attributable to imbalance of hormonal stimuli. The inducing stimulus is a physiological hormone acting it may be, in physiological concentration but unrestrained by balancing hormones. Whether a normal physiological agent such as gonadotrophin should be called a carcinogen is arguable. In many inbred strains of mice, tumours of particular kinds develop with a high frequency approaching 100 per cent, in mice subjected to no experimental interference and suffering from no identified physiological or pathological disorder the carcinogenic stimulus if there be one, seems to be physiological. The unfortunate victims of *Xeroderma pigmentosum* approximate to the inbred mice. The inciting stimulus is, indeed, known but the disease results from an abnormal, genetically determined, sensitivity to that stimulus.

No satisfying synthesis of the oppressive mass of information about carcinogenesis is available or perhaps, now possible. The virus theory and the mutation theory survive in modified and attenuated forms. It is accepted that viruses can induce neoplasia and that they probably do so *more frequently and variously at least under laboratory conditions*, than formerly supposed, but how often they do so under natural conditions in animals or more important, in man is unknown. Furthermore it is not known whether or not the more recently studied virus tumours conform with the essential presumption of the virus theory as advocated twenty years ago that the virus not merely induces neoplasia but persists and *determines the whole course of neoplasia and all its properties*. I do not *know of any recent formulation of the virus theory that attempts to apply* it comprehensively and in detail to the origin, development and clinical behaviour of neoplasia as it occurs naturally in man and animals. The mutation theory is fool-proof provided that the meaning of the term mutation be modified and qualified to meet new needs as they arise. Neoplasia certainly involves heritable cell changes whether or not they *are appropriately called mutations is another matter* Without detailed clarification and application neither the virus theory *nor the mutation* theory is of much more than polemic interest. The need is *for* factual information about what happens in cells that become neoplastic. Accepting mutation in its most elastic sense for any heritable change it remains to find out what in the cell changes how it is changed and how the results of the change are expressed in neoplastic behaviour The filterable agents of fowl tumours *offer one approach to these problems* chemical carcinogenesis offers another

The current trend is to interpret chemical carcinogenesis *in* terms of chemical action of the carcinogen on cell proteins or nucleoproteins. It is

suggested, for example, that carcinogens combine directly with key proteins essential for the control of growth (Miller & Miller 1953), that they act by deleting gene proteins and interfering with gene synthesis (Haddow 1958) or that they bring about a loss of enzyme systems concerned in specific cell functions (Rusch, 1954, 1956). The concept is one of material deletion, the consequent abnormality of cell behaviour being attributed to an incapacity to respond to normal growth-regulating mechanisms rather than to an acquired ability to overcome those mechanisms. This is inherently reasonable. Neoplastic cells probably have nothing new save the negative characteristic of *growing out of harmony with the rest of the body*. The proliferative capacity is not new: no carcinogenic procedure confers neoplastic properties on cells that cannot proliferate. The current deletion hypothesis is different from the old but lingering idea of 'reversion to embryonic type'. There is no reversion. The resemblance between neoplastic cells and embryonic cells is much the same as that between the second-childhood of a senile man and real childhood: and everyone knows what that amounts to.

Green (1954, 1958*a*, *b*) has advanced an elaboration of the deletion theory incorporating a supposition, expressed by others in various forms, that the normal growth regulating mechanism depends on antigen-antibody relationships between cells. According to Green's immunological theory, which does not lend itself to concise exposition, normal cells contain cytoplasmic tissue-specific antigens ('identity protein' complexes) by which the growth-regulating mechanisms recognize them. If the cells lose their identity antigen and become unrecognizable the neoplastic state will develop. Green describes several possible mechanisms of deletion. In chemical carcinogenesis he believes that one or more of the identity protein complexes are bound to carcinogen and thereby modified to form self-replicating auto-antigens which elicit non-lethal antibody. Continuous exposure of cells to the non-lethal antibody will induce an adaptative loss of antigen from some cells: these altered cells will then originate a new race of cells which, lacking identity antigens, will be in a greater or less degree unrecognizable to the growth regulating mechanisms. Green presumes that carcinogens induce also an iso-antigenic change that elicits a lethal antibody which, by reason of its less prolonged action, is the more likely to induce adaptation. He suggests alternative mechanisms for the deletion of identity proteins in other forms of neoplasia induced, for example, by hormones or by viruses.

Burnet (1956) favours a comparable immunological theory of cancer based on his own concept of 'self-markers' in place of Green's 'identity proteins'. The evidence in support of these theories is still indirect. Both

theories involve far reaching biological implications that cannot be discussed here. It seems to me a weakness that, as stated explicitly by Burnet, the theories accept, as given, the processes by which the organism grows to its characteristic morphology and leave unspecified the nature of the morphogenetic field that must be supposed to control the process of repair after injury the theories thus exclude from consideration the problems that may well be most germane to the understanding of neoplasia.

My own thoughts have been tending towards a developmental concept of neoplasia, carcinogenesis being viewed as a diversion of cells into new abnormal pathways of development (Foulds 1958c) The diversion may be effected in varied ways and possibly may occur spontaneously by random changes in genetically unstable cells. The diversion of path and progression along the new path involves permanent, heritable, cell changes. Normal embryonic development involves comparable changes whose nature has not been elucidated. Far from reverting to embryonic type, neoplasia advances further and further away from it but in its progression it conforms with many general principles applicable to normal development and may involve similar but as yet unknown mechanisms.

I do not wish to elaborate this personal point of view except to suggest the need to think about neoplasia in a more dynamic way and in terms of developmental processes rather than in terms of lesions and lumps. From evidence given here and in more detail elsewhere (Foulds, 1958b) it is evident that in man as in animals, carcinogenic action induces a persistent state of incipient neoplasia more extensive and diffuse than early clinical signs indicate. The visible lesions, which are often imperfect neoplasias, result from focal progression within the area of incipient neoplasia. Further progression onwards to malignant neoplasia occurs in some of the lesions in some of the patients. The usual path of progression to carcinoma may traverse a distinctive precursor lesion but there are often alternative paths through different lesions and nearly always, it seems, a direct path from incipient neoplasia passing through no precursor lesion at all. The precursor lesions although liable to progression do not inevitably undergo progression and as a rule most of them do not instead, more usually they persist with unchanged characters or regress. Even in Xeroderma pigmentosum by no means every lesion undergoes progression to carcinoma.

Neoplasia needs studying from beginning to end as a dynamic process advancing with a choice of pathways through qualitatively different stages at many of which there is a choice between regression persistence and progression. The natural history of neoplasia in man deserves much more study with more attention to the course of individual lesions. The dynamic

developmental approach offers new opportunities for clinical research on neoplasia of the skin which might be highly rewarding not only in clinical practice but also as a contribution to fundamental cancer research.

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COMPARISON OF THE HISTOGENESIS OF RADIATION AND OF CHEMICALLY INDUCED SKIN CANCER

BY A. GLÜCKSMANN*

The recent progress of radiotherapy and chemotherapy of cancers has transformed the academic interest in tumour biology into matters of practical importance since the type and physiology of the tumour influence the choice and outcome of treatment. Furthermore, the public interest in cancer prevention and in particular the risk from radiation and from smoking have given a new impetus to studies of the nature of carcinogens, their mechanism of action and of the nature of the biological processes induced by them. Finally the establishment of special centres for cancer detection and the attention given to the treatment of cancer in the preinvasive stage are clear demonstrations of the importance of tumour development. Underlying all these advances is the concept that cancers are caused by the action of a variety of agents on normal tissues, that they evolve in stages and differ in their malignancy and curability. The progression to autonomy of hormone-dependent tumours illustrates such stages in tumour development. A great number of physical and chemical agents and some viruses have been recognized as carcinogenic, but the mechanism of their action is still unknown.

The variety of carcinogenic agents is in strong contrast to the rather restricted number of tumour types elicited by them and this fact has encouraged the natural desire to find a factor common to the production of cancers by various means, be it a virus, a gene mutation or some change in the nucleoprotein constitution of the cell. Though the cancer cells may differ from normal cells irrespective of the carcinogenic agent which induced them, the process of carcinogenesis in its early stages and its duration may vary greatly with the carcinogenic agent employed. To describe the stages in the carcinogenesis due to penetrating radiations and to compare them with those following the application of carcinogenic hydrocarbons to the skin, is the subject of this paper.

Before embarking on a discussion of carcinogenesis in the skin, I should like to stress that the epidermis with its appendages such as the hairs and even the dermis are renewal systems which are subject to intricate regulatory mechanisms. In the epidermis the rate of cellular proliferation is linked

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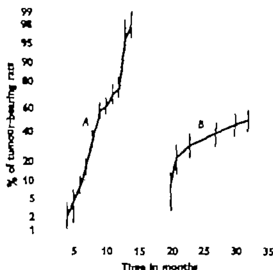
with that of keratinization and of shedding of the cornified material. The removal of the cornified layers by adhesives is followed by a mitotic burst in the basal layer (Pinkus, 1951). In this instance the apparently dead cornified material acts as a regulatory mechanism within the epidermis by inhibiting cell division through a 'feed back' mechanism (Chase, 1954). The skin and hair cycles in rodents are other examples of regulatory changes subject to control mechanisms, the action of which is as yet largely unknown though a new hair cycle can be elicited by simple plucking of hairs. Associated with the skin and hair cycle are periodic changes in the glycogen content of the epithelial structures, the volume of the sebaceous glands and the thickness of the dermis and dermal fat layer (Chase, Montagna & Malone, 1953). Dermal thickness is also known to vary with the oestrous cycle (Bullough, 1943) and this suggests hormonal control. Hyalinization of the dermal connective tissue and atrophy of the epidermis overlying keloidal scars suggests regulation of growth and dependence of structure on the blood supply of the skin. There are thus a variety of regulatory processes at work at different levels. The breakdown of the regulatory mechanisms which adjust the rate of cellular proliferation in relation to differentiation and subsequent loss of cells is one of the characteristics of malignant change in the skin.

Obviously this failure of control may occur at different levels and the various carcinogenic agents may affect different activities. Chemical carcinogens are thought to act more or less directly on the epidermal cells in the 'two stage' concept (Salaman, 1958) of initiation and promotion, and other authors consider the epidermal changes secondary to primary effects on the dermis (Orr, 1958). The primary effect of the carcinogen is being thought of as growth stimulating (Pullinger, 1940; Glücksmann, 1945), as growth inhibiting (Haddow & Robinson, 1937), as modifying or initiating (Berenblum, 1954; Foulds, 1954), or as entirely destructive as, for instance, in the case of irradiation. To become effective, a carcinogenic agent may require a variety of conditions in the animal such as a certain genetic constitution, sex, tissue sensitivity, etc. The action of a given carcinogen may also vary with the dose in which it is given and the period of time over which it is applied. An understanding of these variables can be achieved only by a study of the process of carcinogenesis under various conditions. We shall use merely as illustration the 'direct' action of chemical carcinogens and contrast them with the more 'indirect' action of penetrating radiations.

The results of treatment of rats with a chemical and a physical carcinogenic agent are contrasted in Text fig. 1. The chemical used is 9,10-dimethyl 1,2-benzanthracene (DMBA) applied in acetone solution to the interscapular region once weekly. In the radiation experiments an electron-

beam generated by a Van den Graff accelerator was used allowing a sharp cut off in the depth of the skin and adjustment by changes in the voltage. Skin regions of about 2.5 cm. diameter were exposed to a single dose of 11 000 rads.

Of rats treated with the chemical 98 per cent had multiple malignant skin tumours in 13 months. Radiation induced malignant tumours in only 50 per cent of the rats at risk in a period of 33 months. Similar observations were made with mice weekly applications of a 1 per cent solution of benzpyrene in acetone induced multiple malignant tumours in nearly all



Text-fig. Rates of tumour induction in rats after an exposure to an electron-beam (*A*) and following weekly applications of 9,10-dimethyl-1,2-benzanthracene (*B*).

animals in 5 months, while exposure to an electron-beam induced malignant tumours in only 15 per cent of animals in a period of 20 months. The chemical carcinogens acted more rapidly and caused a greater incidence of tumours than the physical carcinogen. The difference in the results of the physical and chemical treatment is unlikely to be due to a difference in the *timing* of the applications. *Henshaw Snider & Riley* (1949) have shown that for β-rays a single whole-body exposure is more productive of tumours than repeated exposures given over a long time. After the chemical treatment numerous tumour foci arise and become confluent so that tumours counted as single may in fact be the result of the combination of several tumours which have arisen at about the same time though independently of each other With radiation this phenomenon occurs only rarely and the difference in carcinogenicity is even greater than shown in Text-fig 1

The histological type of tumour induced varies with the site in the body with the agent and with the manner of application. DMBA induced in th skin of the interscapular region 73 per cent carcinomas and 27 per cen sarcomas, in the skin of the vulva region only carcinomas and in the vagin only 4 per cent carcinomas and 96 per cent sarcomas (Text-fig 2). With radiation the depth of penetration appears to be of importance the electron beam used in our experiments on the interscapular skin induced 14 per cen carcinomas and 86 per cent sarcomas. Applying a β ray source to th surface of the skin resulted in the appearance of carcinomas and sarcomas in equal proportions (Passonneau & Hamilton, 1951). Subcutaneous implantation of such sources increased the proportion of carcinomas to 61 per cent and intraperitoneal implantation to 84 per cent with corre



Text-fig 2. Proportion of carcinomas (black column) to sarcomas (partly open column) in rats after different treatments and at different sites (A) Dorsal skin painted with DMBA. (B) Vulva painted with DMBA. (C) Vagina painted with DMBA. (D) Dorsal skin exposed to an electron-beam. (E) Dorsal skin exposed to a subcutaneous β-ray source. (F) Dorsal skin exposed to an intraperitoneal β-ray source. (G) Dorsal skin exposed to a superficial β-ray source.

sponding decreases in the proportion of sarcomas (Schubert, Künkel et al. 1956). A possible explanation of these differences may be looked for in the persistence of hair follicles which act as one of the sources of carcinoma formation (Lacassagne & Latarjet, 1946 Wolbach, 1951) and the presence of a very cellular dermal scar tissue which would favour the formation of sarcomas.

There may be some more intricate biological mechanisms which determine both incidence and types of tumours even after the application of powerful chemical or physical carcinogens for instance, the incidence of tumours in the vagina induced by painting with DMBA, is reduced by ovariectomy which, however does not affect the rate of tumour production in the vulva. Injections of oestradiol into castrated rats slightly increase the tumour incidence in the vagina. This shows that the hormonal situation in the animal influences the tumour induction even by powerful carcinogens

The tumour type can also be modified by various means. Thus application of DMBA to irradiated skin favours the production of sarcomas instead of carcinomas which predominate after the application of DMBA to normal skin. There are thus differences in incidence of tumours and in the tumour type induced, with type of agent and with varying local and hormonal factors in the animal. Does the study of the histogenesis of these tumours shed any light on these problems?

The normal skin of rats and mice has a very thin epidermis covered by a thin layer of keratin. The thickness of the epidermis, of the dermis and dermal fat layer vary with the stage in the hair cycle (Pl. 1 fig 1)

Treatment of mice with benzpyrene is followed even after the first painting by marked hyperplasia of the epidermis which extends to the root sheath of the hair follicle and varies in extent with the stage of the cycle. Sebaceous glands undergo a squamous metaplasia. Usually there is only a slight inflammatory reaction in the dermis. Soon after painting the number of mitoses in the epidermis increases while only a few cells degenerate. Basal like differentiating prickle cells enlarge in volume and the balance between proliferation and differentiation in the epidermis is shifted in favour of the former The extent of these hyperplastic changes varies with the phase of hair cycle and quantitative studies reveal peaks of hyperplasia recurring after periods of about 21 days (Glücksmann, 1945).

About 2 months after the beginning of treatment, that is during the third hair cycle, the growth of hairs is abnormal and accompanied by inflammatory changes in the superficial dermis which shows marked fibril formation. At the fourth or fifth hair cycle the abnormal growth of hair follicles results in the appearance of warts which, like the epidermal hyperplasias, vary in degree with field in the skin. The changes occur simultaneously throughout affected structures such as hairs and the epidermal regions between hair follicles, and they do not look like the out come of the multiplication of single affected cells. The same applies to the next stage, namely the appearance of invasive carcinomas in hyperplastic or papillomatous regions. This step brings about not only a further shift in the balance between proliferation and differentiation but also a series of qualitative changes in differentiation, cohesiveness, motility invasiveness, immunology etc., of the cells. It occurs about 10 weeks after the beginning of the treatment.

It is not clear whether the hyperplasia seen after painting with a full carcinogen is of significance for the future development of malignancy or whether it is simply a by-product, since some initiators like urethane are supposed to act without inducing such hyperplasia this can be evoked in mice by croton oil which is only a weak initiator Without going into the

question of the significance of the hyperplasia, it is a convenient way to the undoubted change in the reactivity of the treated cells.

The initial changes induced by chemical carcinogens in mouse skin be summarized as stimulation of proliferation combined with inhibition delay in differentiation. The manifestations of these effects are hyperp of the epidermis and hair follicles with abnormal regrowth of culminating in the appearance of warts. In some of these lesions invasi squamous cell carcinomas arise but their appearance represents a qualitati change from the precursor lesion as regards autonomy and co-ordinatio of cells their differentiation, motility and variability amongst other fea

The changes induced in mice by exposure to a dose of 8000 rads an electron-beam are very different from those just described. This dosag is optimal for tumour production and causes epilation, a radiation b and subsequent formation of unstable scars the edges of which undero malignant change. Histologically the epidermis thickens through an crease in volume of cells and a shift in the balance between proliferating a differentiating cells in favour of the latter (Pl. 1 fig. 2). Indeed thro' the loss of basal cells, cornified cells shed normally from the surface are replaced and thus the skin is denuded. Similar effects lead to epilation to the failure of hair follicles to produce new hairs. Concurrent vascul and dermal changes lead to the appearance of the burn (Pl. 1 fig 3) Demarcation of the dead tissue which is confined to the dermis epidermis (Pl. 1 fig 4), is slow to start and is followed by a formation a thin fibred cellular scar and covered by epidermal cells migrating from the periphery into the lesion. This epidermal regeneration may lead to degree of hyperplasia (Pl. 1 fig 5) and attempts at formation of hai follicles, particularly if the skin is treated with croton oil subsequent to radiation (Pl. 1 fig 6). The scar which remains cellular and thin-fibred breaks down and with it the regenerated epithelium and the lesion is demarcated forming a new scab Under it, cells immigrating from the adjacent epidermis grow directly over the dermal fat layer (Pl. 1 fig 7) in the complete absence of dermis and dermal scar The deeper tissues that is the dermal fat layer the panniculus carnosus and the deeper subcutaneous layers and blood vessels are now involved in scar formation while adjacent dermal tissue fails to participate. The vacuum left by the demarcation of the initial dermal scar is filled by the bulging upwards of the deeper underlying tissue (Pl. 1 fig 8).

The subsequent scars which are formed in the absence of dermal contributions show a progressive tendency to become avascular (Pl. 2 fig 1) undergo lysis and to form blisters which lift the epidermis from its support and cause it to degenerate (Pl. 2 fig 2). The lysis of the scar

proceeds in the almost complete absence of inflammatory cells (Pl. 2, fig. 3). In the periphery of the lesion the epidermis becomes hyperplastic (Pl. 2, fig. 4) and shows downward projections some of which may become malignant and invade the deeper tissue (Pl. 2, fig. 5). They may also rise and form warts (Pl. 2, fig. 6) and from these regions may arise invasive squamous cell carcinomas (Pl. 2, fig. 7) which involve the panniculus carnosus or its remains. Such changes occur after about 14 months.

Thus with radiation the process of carcinoma formation is secondary to the effects on the dermis and the production of an unstable scar. The remarkable absence of dermal regeneration needs emphasis. In mechanical trauma the major contribution to regeneration of the skin comes from the accumulation of cells at the border of the dermal lesion. If radioactive sulphate is injected intraperitoneally it is found that just these cells give a very strong autoradiograph and are mainly responsible for the formation of fibres in the regenerating tissue. Radioactive sulphate is transferred from the cells to the ground substance prior to fibre formation (Glückmann, *et al.* 1956). The deeper tissue of the subcutis which bulges upward upon the excision of the dermal layer provides only a preliminary dressing together with the fibrinous exudate from the blood vessels and thus takes up little sulphate and contributes only little to fibre formation in the scar. After exposure to radiation the dermal component for regeneration is usually missing and the deep blood supply is also progressively affected by radiation, and in view of these failures no satisfactory scar is formed.

That the emergence of carcinomas is linked with the depth and degree of the dermal lesion is seen in further experiments in which only the superficial portion of the dermis with the epidermis are penetrated by an electron beam. No tumours follow. Similarly with smaller doses of radiation or smaller fields of exposure, regeneration tends to be successful and no tumours result, whilst with larger doses of radiation the attempts at regeneration are frustrated and again no tumours follow. Thus the tumours induced in the mouse epidermis by irradiation occur in regenerating rather than in directly exposed tissue and are dependent on an optimal balance between injury and repair.

There are significant differences between the mouse and the rat in the reaction to irradiation and to carcinogenic hydrocarbons as regards the time scale, the greater reactivity of the dermis of the rat as compared with that of the mouse and the different behaviour of the hair follicles. The changes in the rat are relatively slow as compared with those in the mouse and this is particularly striking for the chemical carcinogen. While the mouse epidermis responds to benzpyrene, the rat epidermis fails to do so and

therefore DMBA instead of benzpyrene has to be used. The paintı induces a hyperplasia in the epidermis and the hair follicles (Pl. 2, fig. very similar to that observed in the mouse, that is, a shift in favour proliferating cells at the expense of differentiating and cornifying cel The process develops, however much more slowly in the rat than in tl mouse (Pl 3 fig 1) As in the mouse the hyperplastic change may al proceed to papilloma and wart formation (Pl. 3 fig. 2). The hair follicl of the rat react to the chemical carcinogen with a multiplication of the bas cells which leads to the development of a basal-cell type of tumour (Pl. figs. 2 3) The papillomas, regions of hyperplasia and the basal c tumours arising from the hair follicles suddenly undergo a malignant chan which is accompanied by a loss of cohesiveness of cells of the ability differentiate fully of uniformity in the cellular reaction and by invasi growth (Pl. 3 fig 4).

Another difference between mouse and rat is the production in the latt of a very cellular thin fibred scar tissue in the dermis which tends t undergo a sarcomatous change fairly suddenly and under conditions not yı understood (Pl. 3 fig 5).

The rat thus appears to differ from the mouse in the greater reactivit of the dermal connective tissue to carcinogenic hydrocarbons and in rather reduced responsiveness of the epidermis. This is supported by th fact that the carcinogenic agents for mouse epidermis are far more numerou than those active on the rat skin, while the reverse holds for the substance able to induce sarcomas by subcutaneous implantations.

The rat skin reacts to radiation also somewhat differently from the mous skin The early changes are very similar in both species. A radiation burr develops slowly and gradually involves deeper tissues and the deeper blood vessels. The rate of demarcation of burned tissue after radiation is de creased by the progressive extension of the damage into the deeper tissues and the progressive involvement of the deeper blood vessels which cuts off the supply of white cells for demarcation. There are thus usually a number of abortive zones of demarcation (Pl. 3 fig 6) apparent during the first and second month after radiation. The first one is superficial and later zones develop in regions which subsequently die and thus inhibit further immigration of white cells. After about 2 months all the tissue which has been injured is being demarcated and scar formation starts. As in the mouse the scars are unstable and this leads to the appearance of atrophic, keloidal (Pl. 4, fig 1) and of cellular thin fibred (Pl. 4 fig 2) scars. These different appearances are presumably different stages in the same process. Even if the scars are not actually breaking down, they are con tinuously changing Resorption of old fibres and new fibre formation can

be observed in autoradiographs for long periods after exposure. Originally thin-fibred cellular scars may become keloidal and later atrophic, and then be replaced by a more cellular scar which may give rise to the formation of sarcomas in either the pannicular (Pl. 4, fig 3) or the superficial region. They usually appear close to ulcerations, which are due to the vascular damage which can be roughly described as a spreading endarteritis obliterans and periphlebitis (Pl. 4, fig 4). At the periphery of these ulcerative regions sarcomas and carcinomas emerge in relation to unsuccessful attempts at regeneration and scar formation (Pl. 4, figs. 5 6).

In the rat as in the mouse the progressive vascular changes associated with unstable scars are responsible for the development of malignant tumours. In the mouse the scar becomes avascular and acellular and under goes lysis. This phenomenon is not seen in rats and it may have an influence on the development of sarcomas in rats and carcinomas in mice. Whether the progressive vascular changes are entirely characteristic for a radiation burn is difficult to decide. Even repeated thermal burns fail to produce similar vascular changes and fail to induce tumours in rats.

The histogenesis of tumours produced in rats and mice by the application of carcinogenic hydrocarbons and by an electron beam shows that the mechanism of tumour formation is different. Carcinogenesis caused by radiation is a more indirect process than that induced by hydrocarbons. Radiation kills the treated cells, produces progressive vascular lesions and through these affects scar formation and epidermal regeneration. The instability of the scars leads to repeated attempts at regeneration and malignant change occurs mainly in the immigrating regenerating tissue. It is thus not unlikely that few if any of the ancestors of the malignant cells have been actually exposed to radiation. The process induced by radiation is certainly not simple and direct, and there is no evidence either histologically or from the physical factors of exposure for an immediate mutation of individual cells to become malignant. Most of the irradiated cells are shed in the slow development of the radiation burn.

These differences in the point of attack of radiation and of chemical carcinogens may account for the differences in induction time and in the incidence of tumours. The more direct effect of the chemical carcinogens explains the shorter induction time, and as regards tumour incidence the association of carcinogenesis with a sufficiently disturbed process of re generation lowers the efficiency of radiation. Injury and repair depend on dose of radiation, on the size of the field of radiation and on the depth of penetration, and these factors determine an optimal dose range for cancer induction bigger or smaller doses produce fewer tumours.

As regards the tumour type some obvious quantitative factors may be

involved in addition to the more complex pattern of species and tis specificity Carcinomas arise from the epidermis as well as from the h follicles and the absence of hair follicles in the vagina or after epilatory do of radiation favours the development of sarcomas instead of carcinom Thus application of DMBA to an irradiated rat skin results in the emerger of sarcomas rather than carcinomas, and the absence of hair follicles a the cellularity of the dermal scar combine to shorten the induction peri for sarcomas from 11 months to 9 months and to lengthen that for carcinom from 9 months to 11 months.

The differences between the rat and the mouse in their reaction carcinogens also have a quantitative aspect, that is the greater density of t hair coat in the mouse than in the rat and the thicker dermis in the rat compared with the mouse. From a purely quantitative point of view the factors would favour the development of carcinomas in mice and sarcom in rats Depth of the lesion combined with epilation may account for th production of sarcomas in rats with an electron-beam and of carcinom with the intraperitoneal implantation of radioactive sources.

While these observations help us to understand some of the conditior preceding and facilitating the malignant change, we have not come an closer to explaining the actual process of malignant conversion and of th factors determining it. The term malignant conversion or whatever oth term we use is still only descriptive and the conditions that are responsibl for the sudden change of the hyperplasia of the benign or dependent tumour into autonomous, invasive and malignant cancers are still unknowr This final break away from the normal regulatory mechanisms may b primarily a failure of the cells to respond to the regulatory stimuli or failure in tissue regulation or both. Indeed as long as we fail to understan the intricate control mechanisms and the responses to them we are likel to fail in our understanding of the final step in the malignant process.

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Fig



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Fig. 3



Fig. 4

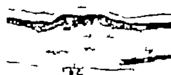


Fig. 5



Fig. 6

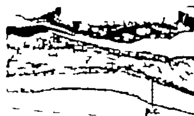


Fig. 7



Fig. 8

For explanation see pp. 3 3 4

(*Facing p.* 332)

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Fig. 1



Fig. 2



Fig. 3



Fig. 4

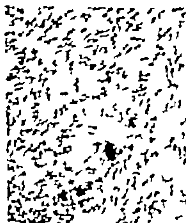


Fig. 5



Fig. 6

For explanation, see pp. 354–5

PLATE 4

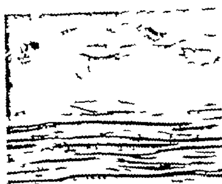


Fig 1

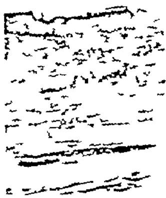


Fig 2

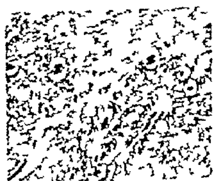


Fig 3

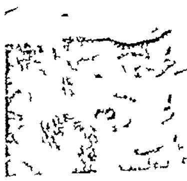


Fig 4



Fig 5



Fig 6

For explanation see p

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EXPLANATION OF PLATES

P TE

The magnification of the original microphotographs are given. For reproduction these were reduced by third.

Fig. 1 Section through the skin of an untreated rat. The dermis appears dark. The hair follicles extend through the dermal fat layer to the dermal muscle (panniculus carnosus) and have reached their maximal length (Anagen). During Catagen, Telogen and the early stages of Anagen the hair follicles are shorter and both the fibrous and the adipose layers of the dermis are thinner 35

Fig. 2. Mouse skin exposed to an electron-beam 7 days prior to fixation An increase in volume of prickle and of cornifying cells has increased the thickness of the epidermis. The hair follicles are short (Telogen) and the adipose layer has almost disappeared. The dermis and panniculus carnosus appear unaffected Dose 8000 rads 00.

Fig. 3. Mouse skin exposed to an electron-beam 5 days before fixation. The epidermis has been shed and the dermis presents burnt appearance the panniculus carnosus is not involved. Dose 8000 rads. 70

Fig. 4. Mouse skin exposed to an electron-beam 5 days before fixation. The burnt epidermis and superficial dermis ha been shed and demarcation of the burn has developed in the deeper parts of the dermis. There is also some round-cell ( ) infiltration in the dermal fat layer The panniculus carnosus (p c.) is hardly involved. Note the regenerative hyperplasia in the adjacent epidermis and hair follicles. Dose 4000 rads. 44.

Fig. 5. Mouse skin exposed to an electron beam 47 days before fixation. The lesion is covered by hyperplastic epidermis and thin-fibred scar tissue. Note the remnants of hair follicles at the left and downward projections of the epidermis related to them. The dermal fat layer is infiltrated, the panniculus carnosus (p) is not involved. Dose 8000 rads. 40.

Fig. 6. Mouse skin exposed to an electron-beam 57 days before fixation and painted weekly with croton oil after exposure. Note the epidermal hyperplasia and the of warts by regenerating hair follicles. The dermal scar is thin-fibred and cellular *dermal fat* layer *is infiltrated; the panniculus carnosus is not involved*, but lar tissue has increased in thickness. Dose 4000 rads. ×40.

Fig. 7 Mouse skin exposed to an electron-beam 98 days before fixation. The or scar tissue has been shed and still adheres to the skin as part of the scab. Hyperp- epidermis has grown under the scab into the lesion and lies directly on top of the fat layer No dermal tissue or dermal scar tissue intervenes between the fat and epidermis. The panniculus carnosus (*p.c.*) is infiltrated and pushed upwards by subpannicular that is subcutaneous tissue. Dose: 8000 rads. ×40.

Fig. 8. Mouse skin exposed to an electron-beam 9 days before fixation. A second is formed by the subpannicular connective tissue which is thin-fibred and infiltrated. panniculus (*p.c.*) can be seen on the left bulging upwards towards the epidermis. epidermis is hyperplastic. There is no participation of the adjacent dermis in scar tion. Dose 8000 rads. ×40.

PLATE 2

Fig 1 *The scar tissue formed by the subpannicular connective tissue has* hyalinized, avascular and almost acellular It forms the main part of the scar tho. superficially some dermal scar tissue is separated from it by the panniculus carnosus (*p.* The epidermis *is* hyperplastic. The dark dermal scar contrasts with the subcutaneous which appears almost devoid of structural details. Fixed 10 months after 8000 rads. ×40.

Fig 2. The subpannicular scar tissue extends right up to the surface, is hyalin avascular almost acellular and is undergoing lysis in the centre. The epidermis is l off by the formation of blister and is degenerating The panniculus carnosus (*p.c.*) the subepidermal region on the left border of the hyalinized scar Fixed 7 months af dose of 8000 rads. ×40.

Fig 3. Shows the central part of Fig 4 at higher magnification. The epidermis is l off by blister from the thickened basal membrane. The lysis of the hyalinized scar proceeds in an almost complete absence f inflammatory cells. oo.

Fig. 4. This scar too is undergoing lysis and has an inadequate vascular supply Most the vessels (*V*) form ring at the periphery of the lesion and few penetrate into it. A margin of the lesion the epidermis is hyperplastic and projects downwards into folds the thickened basement membrane. The dermal muscle fibres are separated by f tissue which merges with the thickened subpannicular zone. Fixed 14 months after of 8000 rads. 40.

Fig 5 An invasive squamous-cell carcinoma has developed in the hyperplastic region the periphery of the lesion. Fixed months after dose of 8000 rads. 40.

Fig. 6. The central part of the scar on the left is formed by atrophic hyalinized and al avascular tissue. The periphery of the lesion is clearly indicated by the conglomeration elastic fibres ( ) in the superficial parts of the dermis. The hyperplastic epidermis proceeded to the formation of papilloma at the periphery of the scar The bulging wards and considerable thickening of the subpannicular tissue increase towards the of the lesion. This is underlined by the dark layer of subpannicular elastic fibres. F 8 months after 8000 rads. Panniculus carnosus (*p.c.*).

Fig. 7 A squamous cell carcinoma fills the burned region Fixed 19 months aft 8000 rads. × 5

Fig 8. Hyperplastic changes in the rat epidermis and in the hai follicles due to the liferation of the basal cells characterize the effect of painting with DMBA I the subep dermal region the dermis has formed thin-fibred cellular connectiv tissue hat appears lighter than the original larger collagen bundles of the deeper fibrous zone. Fixe days after beginning treatment. Total number of paintings 3. 65

PLATE 3

Fig 1 Rat skin 7 months after weekly paintings with DMBA. The proliferation of th cells in hair follicles and of the germinativ layers in the epidermis are learly seen. N the formation of the thin-fibred cellular connectiv *tissue in the superficial parts of* dermis and the abnormal shape of the enlarged hair follicle 5

Fig. 2. Another rat skin 7 months after weekly paintings with DMBA show the formation of papillomas in the hyperplastic regions of the epidermis and on the left the formation of basal-celled (*b*) tumour in hair follicle. The superficial parts of the dermis are formed by thin-fibred cellular tissue. × 40.

Fig. 3. A basal-cell tumour replaces hair follicle in rat skin subjected to weekly paintings ith DMBA for 7 months. × 60.

Fig. 4. Shows well differentiated squamous-celled carcinoma invading the panniculus carnosus (*p.c.*) of rat skin painted weekly with DMBA for 8 months. 20.

Fig. 5. A sarcoma invades the panniculus carnosus of rat skin painted weekly with DMBA for 8 months. Persisting muscle fibres are seen in the lower third of the picture. ×15.

Fig. 6. The periphery of radiation burn in rat is seen 28 days after dose of ,000 rads. The subpanniculer (S) tissue is oedematous, thickened and bulges upwards. The ssels of this region are dilated. The dermal fat layer is infiltrated by inflammatory cells and the lower parts of the fibrous dermis appear to be burned but are not yet demarcated from the living tissue. Above this zone are at least two zones of partially demarcated dead tissue (a). × 20.

PLA 4

Fig. 1. A keloidal phase in radiation scar of rat 27 months after single exposure to ,000 rads. The muscle fibres of the panniculus carnosus are separated by fibrous tissue. 1 0.

Fig. 2. A cellular thin-fibred stage in radiation scar of rat 5 months after exposure to ,000 rads. × 0.

Fig. 3. A sarcoma involving the panniculus carnosus of rat exposed to dose of ,000 rads. 30 months before fixation. Persisting muscle fibres appear as shrunk dark structures. × 70.

Fig. 4. Vascular changes in the form of endarteritis obliterans with perivascular infiltration and fibrosis in radiation ulcer of rat 28 months after exposure to dose of ,000 rads. The epidermis and superficial dermal scar have been shed. 60.

Fig. 5. Papillomas arising at the edge of radiation ulcer in rat 32 months after exposure to dose of ,000 rads. 45

Fig. 6. A squamous-cell carcinoma arising at the periphery of radiation ulcer in rat 20 months after exposure to dose of ,000 rads. × 45

ENVIRONMENTAL SKIN CANCER WITH SPECIAL REFERENCE TO MINERAL OIL CARCINOGENESIS

By D. L. WOODHOUSE

ENVIRONMENTAL CANCER

Many factors are involved in the genesis of cancer in some instances know that exposure to certain well-defined chemicals will induce the cerous changes in the cell with which the pathological condition is Thus is specially so with regard to cancer of the skin. Ever since civil have become dependent on power-industry man has been subm himself to increasing contact with chemical substances which induce canc for many of these are associated with the products of combustion, essent derived from coal and oil, that is the agents are environmental and occupational.

We are particularly concerned with oil, but since the responsible stances from this source are likely to be closely allied to those from coal should be mentioned that a great deal of our knowledge and interest chemical carcinogens stems from the observation of Pott (1776) the connection of soot with the skin (often scrotal) cancer of the chimne sweep of his day Table 1 shows some landmarks in the discovery environmental cancer

Table 1 *Discoveries of occupational cancers of skin*

Agent	Date	Discoverer
Soot	1775	Pott
Arsenic	1822	Paris
Coal tar	1876	Volkmann
Paraffin oil (shale oil)	1876	Bell
Shale lubricating oil	9	Wilson
Creosote oil	900	O'Donovan
Petroleum lubricating oil	930	Heller

POLYCYCLIC HYDROCARBON CARCINOGENS

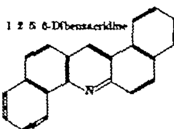
Following the production of indisputable cancer by coal-tar application to the skin of rabbits and mice by Japanese workers (1915 18) great were made to identify the agent(s) responsible. It was shown that activi was concentrated in the higher boiling fractions and due to neutral pounds containing only carbon and hydrogen. Around 1925 Kennaway produced cancer-inducing tars by passing acetylene and other

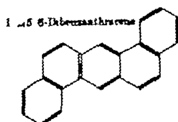
hydrocarbons with hydrogen through heated tubes containing catalysts. A complex mixture of hydrocarbons, including polycyclic types, is formed and some of them are cancer inducing This process imitates on a small scale processes which occur during some stages in the generation of mineral oils in the earth and the molecular rearrangements occurring during certain operations in the processing of crude oils.

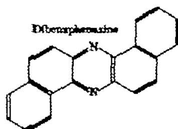
Using as a guide the fluorescent spectra of such synthetic tars, those of coal-tar extracts and those of some synthetic benzanthracene compounds (Hieger 1930), it was demonstrated by animal tests that the polycyclic hydrocarbon, 1:2,5'6-dibenzanthracene, was a powerful skin carcinogen, and in 1933 (Cook, Hewett & Hieger) a particular ingredient of coal-tar 3,4-benzpyrene, was shown to be even stronger (Fig 1). These substances are, however only two of a large number of cyclic hydrocarbons which have been synthesized and which when tested on rats, mice and other animals show varying degrees of activity Applied to the skin of mice in solvents such as acetone or alcohol, benzpyrene will induce papillomas in about 10 weeks using twice weekly applications of about 1/10 mg., and practically 100 per cent of animals surviving 30 weeks will exhibit epitheliomas. If a few milligrams are injected into the subcutaneous tissue in a suitable vehicle sarcomas develop after a similar period Many of these compounds were synthesized by J W Cook and his associates.

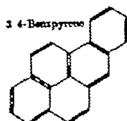
As well as this type of polycyclic hydrocarbon, containing only carbon and hydrogen, there are some active related substances in which an oxygen, nitrogen or sulphur atom replaces a carbon in one of the rings (Fig 1). Also a fused ring system with relationship to certain physiological steroid substances is present in a very powerful carcinogen, 20-methylcholanthrene (Fig. 1). It is possible that allied sulphur or nitrogen-containing carcinogens may occur in mineral oils in small amounts. Further information on various aspects of chemical carcinogenesis is given in the books mentioned in the bibliography

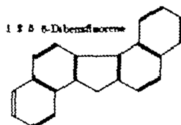
It is not known definitely whether benzpyrene itself is contained in crude mineral oils or indeed whether their carcinogenicity is due to any of the above types of compound. It is quite definite, however that small amounts of such chemicals are produced during industrial cracking processes, in which the molecules of the heavy oils are broken down or rearranged, thus providing greater amounts of more valuable, volatile types of hydrocarbon for fuels, lubricants or further chemical processes. Similarly small amounts are produced when hydrocarbons are incompletely combusted or when subjected to heat during use as lubricants or quenchers.

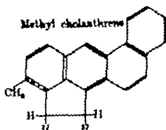

1 2 5 6-Dibenzacridine
N

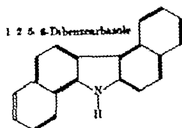

1 2 5 6-Dibenzanthracene


Dibenzphenazine
N
N


3 4-Benzpyrene


1 2 5 6-Dibenzfluorene


Methyl cholanthrene
CH_3


1 2 5 6-Dibenzcarbazole
N
H

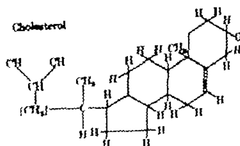

Cholesterol

FIG.

OTHER CARCINOGENIC AGENTS

There are other carcinogenic skin agents such as arsenic, ultra-violet radiations, including sunlight, and radioactive substances, and also there are chemicals which produce cancers in other sites, such as bladder cancer induced by β naphthylamine and allied compounds, but these are beyond the scope of our present interest.

MINERAL OILS

The uses of materials *from mineral oils* are vast and widespread. In addition to direct use as fuels, solvents and lubricants there are extensive outlets in the fields of plastics, synthetic rubbers, fibres, detergents, etc.

The chief components with cancer-inducing activity are usually considered to accompany almost entirely the higher boiling point materials.

Tables 2 and 3 show the annual production and use of some classes of these potentially active materials in the United Kingdom in 1957

Table 2. *Production of high boiling-point mineral-oil products United Kingdom*, 1957

	Thousand tons
Fuel oil	0,500
Lubricating oils and greases	826
Bitumen	8
Paraffin wax, scale and slack wax	28

Table 3 *Use of lubricating oils and greases. United Kingdom*, 1957

	Thousand tons
Aviation	
Industrial	470
Marine	7
Motor	238
Tractor	35
Total	826

According to modern evidence the crude oil now present in underground pockets or lakes has resulted from the decomposition under pressure of the organic remains of plankton and other marine life with possibly some contribution from plant forms. A very high proportion of it consists of hydrocarbons. The gas which is partly dissolved under pressure or in pockets over the more liquid oils consists of open-chain saturated aliphatic and unsaturated olefinic hydrocarbons. The liquid oils vary greatly in their admixture of larger molecules of saturated and unsaturated aliphatic, naphthenic (hydrogenated cyclic) aromatic and polycyclic types. Also the

content of sulphur-containing and nitrogen-containing compounds v widely The more complex molecules occur in the higher boiling fract

Oil refining is one of the triumphs of modern chemical engineeri industrial chemistry Not only does it deal with incredible quantities, does so with minimum of labour and maximum utilization of all prod The processes of distillation, extraction and processing are carried highly automatic lines so that hazards are relatively low It is in the indus

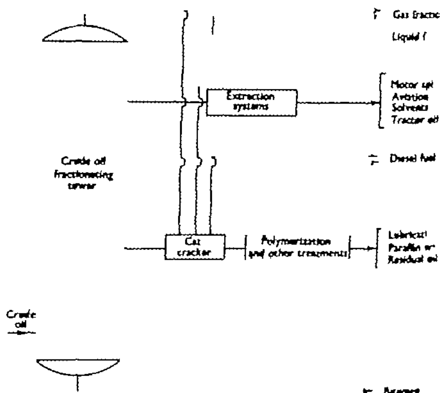


Fig 2. Crude oil fractionation (schematic).

using the products and in which the potential hazards are less likely to b appreciated that the danger due to contact with cancer inducing sub. arises

There are carcinogenic substances in the higher boiling fractions of crud mineral oils but probably much larger amounts result from the catal cracking processes which are carried out to produce chemicals, fuels an lubricants of high economic value from the less valuable high boil ng po constituents (Fig 2) while small but possibly dangerous, amounts carcinogenic substances are produced when previously innocuous lubricant are exposed to heat or other agents during use in various industries.

A source of lubricating oil which was extensively used earlier in the century in this country is shale oil, particularly produced in Scotland, but also now being developed in America. The oil expressed from the shale along with the higher melting point wax was demonstrated to be carcinogenic, and many instances of cutaneous cancer occurred amongst those extracting and using it. It has been found that the hydrocarbons in the shale are non-carcinogenic, but are converted to active types during the heat treatment for separating and refining it.

MINERAL OIL AS AN ENVIRONMENTAL INDUSTRIAL CARCINOGENIC AGENT

Cancer-prone occupations include boatmen fishermen, engine and crane drivers, engine firemen, cleaners, oilers, metal moulders, rubber linoleum manufacturers, cotton mule spinners, machine tool operators, etc. It has been computed that in U S.A. there are over 5 000 000 workers employed in work with exposure to products of combustion, distillation and cracking of coal, oil and other carbonaceous materials. *In this country high incidence* of cancer among cotton spinners has been known for many years (Southam & Wilson, 1922) while recently liability to cancer of the hand and forearm has been shown to be an occupational hazard resulting from exposure to mineral oils in the engineering industry (Cruickshank & Squire, 1950).

Table 4. *Occupational cancer recorded up to 1952*

Agent	Site	No. in U.S.A.	No. in Europe
Coal-tar etc.	Skin		3 50
Petroleum	Skin	70	29
Shale oils	Skin	7	900
Creosote oil	Skin		40

Table 5 *Epitheliomatous ulceration with fatal cases*

950	195	952	953	954	955
28 (8)	9 ( )	34 ( )	60 (42)	30 (9)	48 (3)

Fatal cases are indicated in brackets.

The scope of environmental cancer is not adequately represented by published figures, for in the past many of the tumours were not recognized as due to an environmental factor and others escaped notification on account of inadequate knowledge. Also many were not recorded for such reasons as reluctance of industry to publicize industry related hazards. The long period between exposure and appearance of the disease often interferes with the recognition of its true causation.

Table 6. *Latent period of occupational cancer of skin*

Agent	Average latent period (years)	Range of latent period (years)
Arsenic	18–25	3–46
Tar	20–24	1–50
Mineral oil	50–54	4–75
Crude paraffin oil	15 18	5–35

In 1919 occupational cancer became a notifiable disease under the Fa tories Act and a clause specified epitheliomatous ulceration due to pitch, bitumen, mineral oil or paraffin or any compound or residue of s

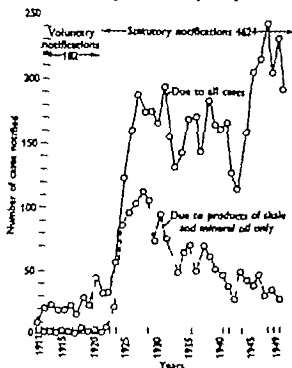


Fig. 3. Coal tar, shale oil and mineral oil cancers of the skin before and after the introduction of Statutory notification in England and Wales (S. A. Henry 1950)

of these substances. Our knowledge of its incidence in this country derive from these notifications although imperfect, the statistics are more complete than those for any other country (Fig. 3).

Occupational cancer may be accepted for benefit under the prescribed diseases provisions of the Industrial Injuries Act if it is shown that it arose as a sequela of any of the prescribed diseases.

It is unfortunate that the reported figures still fall short of the total cases. One of the impediments is the long latent and lag periods while the nature

of the particular job may not be appreciated by the doctor Also the recorded occupation is often the most recent, which may have little relation to an earlier occupation involving exposure to environmental risk. In a recent investigation on 130 consecutive cases of squamous epithelioma treated at one hospital in one year eighty-one had an occupational background, only three of which had been appreciated and reported as such.

MEDICAL AND CLINICAL ASPECTS

It is not the function of this lecture to enter specifically into those aspects of skin cancer According to the literature the following lesions may supervene after exposure to the agents under discussion.

(1) Acute and chronic inflammatory responses (erythroderma, dermatitis folliculitis). Chronic inflammatory conditions *per se* are not carcinogenic, but malignant changes may supervene on such, and physical or chemical trauma to such predisposed skin sometimes precipitate malignant changes.

(2) Pigment disturbances.

(3) Hyperplastic and neoplastic conditions—hyperkeratosis, intra epidermal papules, warts, pedunculated, broad-based papillomas, cutaneous horns—frank carcinomas.

The proliferative manifestations are often multiple and usually on the exposed parts of the skin. Often pedunculated papillomas or dry warts remain benign and in many instances regress. Malignant changes occur in sessile, flat papillomas but cancer may also develop in apparently unchanged parts of exposed skin after a lag period of many years.

RESEARCHES ON MINERAL OIL CARCINOGENESIS

During the period 1923–30 Twort and co-workers at Manchester (Twort & Twort, 1931) in a careful and intensive series of tests, showed that a large number of spindle and other oils were carcinogenic to mice in various degrees. They also indicated that oils originally of low activity are made more potent by cracking and that treatment with sulphuric acid lowers the activity They found marked differences in the activity of oils from the various localities, being high in those derived from Venezuela and Borneo and lower in Texan, Pennsylvanian and Russian derived oils. In an effort to stimulate the use of non-carcinogenic lubricating oils they correlated their tumour-inducing activity towards mice with their specific gravity and refractive index and advocated a specification for a non-carcinogenic oil based on physical qualities. This was, however not found entirely satisfactory and oils complying with a more stringent specification have recently been evolved and made compulsory in spindle spinning (Auld 1950).

My own entry into this field dates to the later war time when the pooling of crude oils from many sources, necessary for economic and military reasons had rendered it impossible to estimate the hazards associated with them. Also residues and various derived materials were being employed in many new ways.

In preliminary work I was able to show on the one hand that many fractions of mineral oils were very potent carcinogens, but that certain grades of white oil of light mineral paraffin type, free from colour and fluorescence, did not produce any papillomas in mice after application for 12 months (Woodhouse & Irwin, 1950) We have since also proved that rabbits are similarly unaffected, which is of importance since some ingredients in mineral-oil fractions affect mice to a very much less extent than rabbits. It is not always safe to argue from animal experiments, but in view of the observations on human incidence we believe that any substance producing even benign papillomas on animals is potentially dangerous to man and that tests to estimate the action of such materials should be made on at least two species (Hieger & Woodhouse, 1952).

The British petroleum industry was able to produce sufficiently large quantities of such an inert white oil of efficient lubricating quality and under the Mule Spinning (Health) Special Regulations (1954) it became a legal requirement to use such lubricants

The use of such safe oils in cutting tool trades presents more difficulty but that a definite hazard is present was shown by Cruickshank & Squire (1950). They found that in a group of 138 employees at bar automatic machines in the Midlands run at high speeds and using large amounts of coolant oil 80 per cent suffered from oil folliculitis, and 50 per cent from warts, while one fatal case of scrotal cancer was encountered. Also, Cruickshank & Gourevitch (1952) in investigating forty four cases of cancer of hand and forearm found that eighteen had a history of extensive exposure to cutting oils. In both sites it is the excess of lubricant thrown off in a fine spray which contaminates the operators. In the case of cutting oils the generation of cancer inducing substances may proceed slowly during the period in which the sump oil is submitted to heat by the cutting operations.

Fortunately it is probable that the incidence of skin cancer in the cotton industry will decrease In the old spindle machines lubrication of the bearing was required very frequently and the spindles ran at over 11,000 r p.m. which resulted in the centrifugal throwout of the excess oil which contaminated the clothing of the attendant whose main duty was to lean over the faller bars above the spindle to piece up the broken ends of thread as they came through the rollers. The mule spindle is specially

useful for finest yarn and in some instances spindle bolsters have been introduced to reduce the numerous oil applications, while sintered graphite bearings have been evolved which require less frequent lubrication. In many modern mills the ring spinner has replaced the mule spinner. Though this does not produce such fine work it is automatic. In 1912 there were 50,000 mule operatives, in 1957 less than 20,000.

These factors, together with better washing facilities in factories and homes and compulsory medical examination introduced in 1954, should do much to eliminate skin cancer in these industries. At present it remains at about one case in 500 working spinners. The late effects of exposure of those who are now in other forms of employment or have retired will still be encountered.

In connection with the subject of personnel protection it has been shown that, for washing, special oil removers are more effective than soap and that, in practice, barrier creams are of limited value.

Since so many different grades of lubricating oil are required for different industries and machines it will be difficult to produce specifications to eliminate all danger. However if the exact chemical structures of the carcinogenic compounds present were known it might be possible to remove them by some economic chemical or physical treatment.

This requires the isolation and testing of the possible carcinogens and a programme of research to this end has been steadily pursued under the aegis to the Medical Research Council for the past seven years.

In the U.S.A. a programme of investigation has also been carried out on oil products, but particularly with the immediate aim of assessing the potency and hazards associated with particular products of distillation, cracking and processing. Thus no precautions have been taken to prevent the generation of new chemical structures either by building up from simpler or breakdown from more complex molecules. An industrial hazard due to mineral-oil products was shown by Smith, Sunderland & Sugiura (1951).

In the British investigation crude oils representative of various types, for example high naphthenic or high sulphur were chosen for study from fields in Kuwait, Lagunillas, and Oklahoma. A preliminary separation of each by distillation was carried out by the Shell Thornton Research Station and from these an extensive series of sub-fractionations and solvent extractions were carried out under the supervision of Professor Morton at the Department of Chemical Engineering, Birmingham University (later at Manchester) (Carruthers *et al.* 1955). The conditions throughout the work have been carefully controlled so as to eliminate any risk of producing carcinogens by cracking. By working under reduced pressure and intro-

ducing steam, fractions were separated containing only the compounds present in the original oil.

These fractions have been tested on mice and rabbits in all cases for a period of 12 months and the results compared with standard solutions of known pure carcinogenic compounds. So far we have been unable to use any physical characteristic as a sure guide in following up the fractionation, for although the crudes and their fractions are highly fluorescent the complexity of the mixture is such as to prevent the spectra or ultra-violet

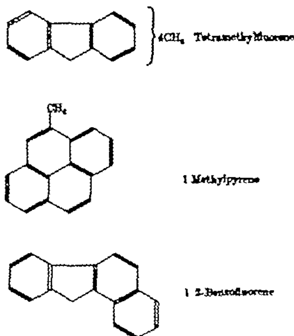


Fig. 4. Compounds isolated from Kuwait mineral oil by chromatography of picrates.

absorption characteristics of any particular component being determined with any precision. The investigations have shown that the crudes contain carcinogenic ingredients and that they are specially concentrated in the high boiling fractions and residues.

The compounds can, however be divided into a number of types, for example, aromatic, aliphatic, anthracenic, phenanthrenic or naphthenic mixtures. Some of these have been separated by extraction procedures, for example of the aromatics by aqueous acetone and these can again be separated into different types by forming complexes with maleic anhydride or picric acid. Separations in special stills and chromatographic techniques have provided materials which Dr J. W. Cook and Dr W. Carruthers at

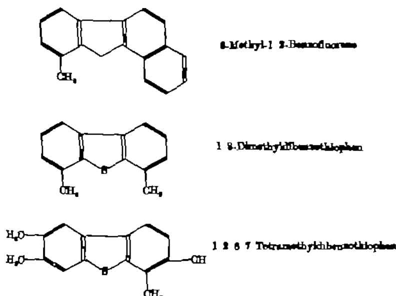


Fig. 5. Compounds isolated from Kuwait mineral oil by chromatography of picrates.

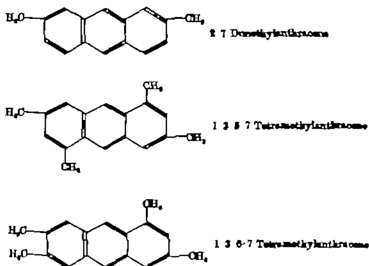


Fig. 6. Compounds isolated from Kuwait mineral oil by chromatography of maleic anhydride extracts.

Exeter are examining to isolate pure chemicals of types possibly cancer inducing. When these have been identified sufficient supplies are being synthesized for tests on animals. A number of those for which the formulae have been proved are given in Figs. 4–6. Amongst those already produced are some containing sulphur. Compounds have been isolated which hitherto have not been tested for carcinogenic activity (Cook, Carruthers & Woodhouse, 1958).

At present the exact biological mechanism by which such hydrocarbons induce morbid cell duplication is not properly understood. Some suggestions based on their reactivity towards cell proteins are finding fairly wide acceptance, while the arrangement of the atoms in the active molecules, compared with structures differing only slightly but showing no cancer inducing activity, is being interpreted in terms of electron distribution.

It is possible that new classes of pure chemical carcinogens will be discovered which will be of interest and use in the more fundamental approaches to the problem of the mechanism of cancer induction.

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THE HAIR CYCLE AND ITS RELATIONSHIP TO EXPERIMENTAL CARCINOGENESIS

By H J WHITELEY

The process of hair replacement in the smaller rodents is discontinuous, occurring in periodic waves of active hair growth which start on the belly and spread over the flanks to the back, with intervening periods of quiescence when there is no growth of hair This process was first described in the mouse by Dry (1926) and in the rat by Haddow *et al.* (1945). A similar pattern of hair replacement has been described in the rabbit (Whiteley & Ghadially 1954), but here the waves of active hair growth, which occur once or twice a year spread from the back to the belly Between these waves of active growth the skin is in a resting state for periods of up to 8 months, and there is no growth of hair In spite of the considerable and expanding knowledge of the structure of the hair follicle and the changes that take place during the hair-growth cycle, which have been extensively reviewed by Hamilton (1951), Chase (1954), Montagna (1956) and Montagna & Ellis (1958), little attention has been directed to the study of the effect of the hair-growth cycle on the experimental production of skin tumours, or how this growth cycle may modify the reaction of the skin to the carcinogen. This is surprising for during the regrowth cycle the follicle epithelium is the most actively dividing and the largest epithelial structure in the skin and there is a well recognized relationship between the mitotic activity of a tissue, in particular skin and the incidence of experimentally produced tumours (Bullough 1950).

While the hair follicle has been suggested as a source of squamous carcinoma in the experimental animal (Glucksmann, 1945) and in man the source of basal cell carcinoma (Foot, 1951), these observations, based solely on macroscopical study of the skin, do not take into account the fact that the hair follicle is not a permanent structure but undergoes profound changes from active growth to almost complete regression. The first observations that the hair-growth cycle might influence the reaction of the skin to a carcinogen were made by Guldberg (1931) when he noticed the fluctuation in the size of tar papillomata parallel with the hair-growth cycle. Similarly Mottram (1945) observed that in mice the size of experimentally produced papillomata fluctuated in association with the hair growth cycle, becoming smaller during the period of hair growth. Wolbach (1951), while unable to correlat the formation and disappearance of

papillomata with changes in the hair cycle, noticed that the cellular activity of the papilloma increased when hair growth occurred in neighbouring follicles. He also observed that the response of the skin to the application of a carcinogen varied greatly in the different stages of the hair-growth cycle.

Andreasen & Engelbreth Holm (1953), in an investigation in which mice in different stages of the hair-growth cycle were painted once only with a carcinogen, observed a higher incidence of tumours when the carcinogen was applied to skin in the resting phase, but no difference was detected in the papillomata that developed from the originally active or quiescent skin. Liang & Cowdry (1955) using whole-thickness mounts of mouse epidermis, were able to show the origin of papillomata from follicles, some papillomata later developing into cancers. They suggested that the differing phases of the hair growth cycle were partly responsible for the differing response of the skin to the carcinogen, and thought that this difference might play an important role in the later stages of carcinogenesis as well as in the early stages. They noticed occasional regression of the narrow-based papillomata, and fluctuation in size of the broad based papillomata with the phases of the growth cycle. Lennox (1955) described the development of tumours from the hyperplastic hair follicles in rat skin following the repeated application of 2-anthramine.

The rat and mouse are not entirely satisfactory animals for the study of the effect of the hair cycle on experimental carcinogenesis, as the period of hair growth is short and the phase of follicular activity is not uniform throughout the flank at any one time.

In an attempt to overcome some of these difficulties the rabbit was selected as the experimental animal partly because of the longer period of hair growth, but principally because it is possible to prepare animals in such a way that known areas of skin are in known stages of the hair-growth cycle at the commencement of the experiment. This is due to the fact that the hair-growth cycle can be artificially stimulated in areas of skin by plucking out the overlying hair during the quiescent phase. Further preliminary investigations (Whiteley & Ghadially 1951) had shown that there was a definite relationship between the first appearances of tumours and the state of the hair cycle at first painting More detailed experiments (Whiteley 1957) in which the flanks of eighteen rabbits were prepared in such a manner that there were areas of skin in the quiescent phase, the 1st day the 12th day and the 30th day of the regrowth cycle at the first application of 1:2-dimethyl-9.10-benzanthracene confirmed the earlier impression that there was a relationship between the hair-growth cycle and the development of tumours. Here two types of tumour were seen to develop in

relationship to the different phases of the hair cycle One type was a histologically malignant tumour occurring during the quiescent phase, that underwent spontaneous regression. The other was a papilloma that developed during the growth phase. Both types of tumour occasionally underwent a change to progressive invasive growth. It was postulated, because of the striking similarity of behaviour between the self-healing tumour and the hair follicle—rapid growth down to the panniculus carnosus and then sudden regression—that the self healing tumour developed from the germinal bud from which the new hair grows. This experimentally produced tumour has its human counterpart in the keratoacanthoma which has been studied in great detail by Ghadially (1958) who concluded that the lesion arises from the hair follicle thus confirming the view of Calnan & Haber (1955) If these tumours develop from the hair follicle in particular the germinal bud, the distribution would be expected in those sites where the resting phase of the hair is long and therefore where the hair is short (Chase, 1954) for the germinal bud would then have a greater length of exposure to the carcinogenic agents that are thought to be partly responsible for its development. This relationship is seen in man, as the lesions are found most commonly on the face, in particular the nose and cheeks (Beare, 1953). This relationship is even more strikingly seen in the distribution of pitch warts (Jenkins, 1948) for out of 158 treated lesions of the head and neck only nine lesions were found in the beard area and one on the hairy scalp The importance of the hair follicle is also shown in the observation that these lesions are not seen on the palms of the hands and Twort & Twort (1936) failed to produce tumours on the soles of the feet of mice Further experiments in progress using rabbits of different hair length indicate that the greatest number of tumours occur on the short haired rabbit when compared with normal and angora strains.

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DISCUSSION

Chairman DR F RAY BETTLEY (London)

BETTLEY Regarding ultra violet light as a carcinogen, it has occurred to me that ultra violet light may be one of the incomplete carcinogens to which Dr Foulds referred perhaps he would comment on that suggestion? This might explain the seemingly rather irregular carcinogenic effects of ultra-violet light on the human skin. I believe Dr Woodhouse hinted at this also and suggested that ultra violet light might be a promoter in a skin that was already injured by tar Men who work with tar become sensitive to ultra violet light and it may be that this sensitivity plays a part in the production of carcinoma.

The substances which sensitise the skin to ultra violet light when they are placed on the skin are often fluorescent or chemically related to fluorescent substances and this makes one recall Dr Woodhouse s mention of the fluorescent properties of the carcinogen. Is there perhaps a relation between photosensitising and carcinogenic properties? After what has been said, clinicians may be a little surprised to see so few skin carcinomas attributable to lubricating oil. This may be because the incubation period is an extremely long one, up to fifty years or more. I wonder whether the carcinogens in the present lubricating oils have always been there? The processes by which crude oil is refined nowadays are different from what they were some years ago and perhaps the old style of lubricating oil contained less carcinogens.

DR J MARTIN BEARE (Belfast). *Has Dr Whiteley been successful in producing the giant form of tumour in the rabbit s skin similar to what we see occasionally on the human skin?*

DR H WHITELEY (Cardiff). The tumours that I produce in rabbits are fairly large and one of them was certainly about three inches across. I do not think it is necessarily the size of the tumour that affects its power of self healing In the rabbit the hairs are in a compound follicle there are large and small hairs and the different sizes of the tumours may depend upon which type of follicle is affected and how many follicles are involved.

DR C H WHITTLE (Cambridge). The question of the distribution of the tumours in man seems very interesting and worth following up *In the picture that Dr Whiteley showed of the tar warts on the face, I think he suggested that it was the lanugo hair distribution which might account for this selection. There is, so far as I know no record of self healing tumours arising on such areas as the palms and soles* where I bel eve there are

sebaceous follicles but no lanugo hairs. Does this fit in with his theory? The only other point is one I have already raised at the last B.A.D. meeting at Cardiff namely the appearance of these tumours on the lip. There are no obvious hairs on the red margin but there are quite definitely sebaceous follicles. Are there perhaps also lanugo hairs? Dr Whiteley took the line, which I do not agree with, that the tumour arose on the hair bearing part of the lip and not on the mucous membrane. The tumour in fact starts—and the residual scar is seen to be—well inside the red margin. It is possible to argue that the tumour started in the hair follicles and emerged from underneath on the surface of the red margin but I think the explanation, which is borne out by other cases in which we have seen the tumour starting on the red margin, is that it does in fact arise on the mucous membrane and develops probably from sebaceous follicles, possibly from lanugo hairs.

WHITELEY I quite agree that there are sebaceous glands in the lip and it is correct that the hair follicles and sebaceous glands form a pilo-sebaceous complex possibly in the vermilion border the sebaceous part only of the complex is present. Whether there are any small or vestigial hairs there I would not like to say but it is a problem that needs investigation. But I still feel the tumour we are now discussing arose from the margin where there are very small hairs, and even if the final centre was in the red margin, I am not sure that this indicates where the tumour originally started. But I agree that this is a point of dispute. There are no pilo-sebaceous complexes on the palms or soles of the feet, and because of this you do not find any keratoacanthomata there.

DR L. B. SNEDDON (Sheffield) I should like to ask Dr Whiteley what the relationship is between his tumours and true epitheliomata. Do they all regress *or do some go on to true epitheliomata?*

WHITELEY I found that about 3 per cent of these tumours do progress and will metastasize. They start in the same way as the self healing tumours and it is impossible to tell the difference when you first see them. Some of them progress and even when there is evidence that they have produced metastases it is impossible to distinguish histologically between them. Even the metastases which are present in the regional nodes seem to undergo partial regression.

DR A. JARRETT (London) In the resting phase of the hair cycle the epidermis and the dermis are also inactive. During the growing phase all three become active and therefore when a stimulus is applied at this time it could equally well induce a normal growth in the epidermal cells as in the hair follicle cells. It is therefore possible that these tumours could originate from the epidermis.

Dr Whiteley mentioned that the growth cycle of these regressing tumours might be related to the natural hair cycle of the animal If this were so one would expect animals with long hair cycles to have long lasting tumours whilst those with short hair cycles would have short lived tumours.

WHITELEY *I have found that the period of growth and regression of the tumour is not directly related to the length of hair The period of growth and regression seems to increase with the duration of the experiment.* I agree that these tumours might arise from the epidermis but I do not think it likely as their behaviour and the relation of the tumours to the cycle suggests that they almost certainly come from the follicles. I do not think regression is related to any change in the dermis, although the dermis itself becomes much more active and the blood supply increases during the growth cycle.

DR N HJORTH (Denmark). Andreasen & Engelbreth Holm (1953) found the incidence of experimental skin tumours in mice to be related to the hair cycle. The problem was further investigated by Klinken-Rasmussen (1956).

It is of interest to note that the Danish workers found the incidence of papillomas in mice to be higher when the carcinogen was applied during the resting phase than after application during the growth phase of the hair follicles.

WHITELEY There is a species difference between the mouse and the rabbit, as regression does not occur in the mouse. This may be partly due to the fact that the growth cycle is much shorter than in the rabbit.

DR C D CALNAN (London) This work on carcinogenesis particularly touches us, as dermatologists, on the subject of promoters. Some recent work on promoting activity of Tweens seems to be of great importance. Tweens are widely used *in cosmetic preparations and one is concerned* to know whether such substances should now be used at all. There is a big difference in the concentrations used. In the experimental work the substances were used pure whereas we use them for skin preparation in very weak concentration. Another point is how far can we apply experimental results in the tumour-susceptible mice to experiments in man? Similar questions have been raised in the case of epoxy compounds which are used in synthetic resin systems. Many industrial scientists are concerned with the question of whether they should continue using them because of their possible carcinogenic activity *I wondered if Dr Foulds would give us his guidance on the line we should take as regards such* substances is there any easy method by which compounds can be assessed *for* their carcinogenic activity before they can be safely put on the market?

DR R. D SWEET (Plymouth) I would be interested to know whether the order of potency of the various carcinogenic agents we have been told about is the same in all species, or whether for instance, one agent might be particularly powerful in the rat only to be surpassed by others, for instance in the rabbit.

I would like to ask Dr Foulds about transplanting tumours from one animal to another one of the same species. Do the same rules apply as with homografts, or are rapidly growing tumour cells no longer antigenic?

DR D L. WOODHOUSE (Birmingham). Different species do respond differently to different carcinogenic agents not only with respect to the skin but also with respect to the production of tumours in other organs. Berenblum & Schoental (1947) obtained a fraction from coal tar which would not induce skin tumours in mice but was active against rabbit skin. The fraction contained no benzpyrene but the chemical responsible was not identified. Also mice are very unresponsive to our mineral oils whereas with rabbits I get all the types of tumours which have been shown on the slides.

DR L. FOULDS (Chalfont St Giles). The justification for using mice for our experiments is that the response is closely similar to industrial experience. As Dr Woodhouse mentioned, you do not always get the same results in rabbits as you do in mice.

About transplantation, I think I must have misled you. The transplantation of tumours can be very rigidly limited just as skin transplantation is, which is the usual experience with a new tumour but in the course of transplantation the tumours are likely to get more adaptable and can be transplanted to more remotely related animals within the same species. You can transplant them to other species by knocking out the regulating mechanism by irradiation or by cortisone but then, as somebody has pointed out, the animal becomes little more than a mobile incubator In mice, generally speaking, you cannot transplant a tumour from one inbred species to another The histocompatibility genes seem to be in the tumour cells as well as in the skin grafts.

DR E. M. DONALDSON (Stoke-on-Trent). What evidence is there that trauma plays some part in inducing skin cancer?

FOULDS. Trauma is a difficult point—there is some ground for regarding it as a promoting agent or a localizing agent. When the skin has been provoked into a state of incipient neoplasia then trauma may determine the point at which a tumour shall arise on the skin.

DONALDSON What are the speakers views on therapeutic tar cancer?

WOODHOUSE. With regard to the use of ointments containing tar extracts, again Berenblum (1947) did some work on this type of substance, using liquor picis carbonis (BP) 20 per cent in alcohol. He applied the solution

for long periods to mice and obtained malignant tumours. I would not like to give an opinion on the effect of such material used therapeutically.

DR S. T. ANNING (Leeds). Is it true that there are carcinogenic substances in exhaust gases?

WOODHOUSE. Much work has been done on this and analogous problems involving atmospheric pollution. Small amounts of substances such as 3,4 benzpyrene have been shown to be present in these exhaust gases. Whether they play a part in evoking lung cancer I would not like to say. The exact physical state of these and other compounds in the air may have an enormous influence. The exact size of the particles, how suspended, and how they are absorbed in the lungs are all factors which have to be considered.

DR T. B. FITZPATRICK (Harvard). Ultra-violet radiant energy appears to play a major role in the pathogenesis of carcinoma of the exposed surfaces in man. The clinical observations are amply supported by studies in which artificial ultra violet radiant energy has been shown to induce the development of squamous cell carcinoma and fibrosarcoma in albino mice. Some recent observations in our laboratory indicate that the rate of tumour induction is significantly less in pigmented (brown or black) mice. This would suggest a protective role of melanin pigment, and explain also the rarity of basal and squamous cell carcinoma in the Asiatic and Negroid races, and the very high incidence of carcinoma of the exposed surfaces in human albinos residing in South Africa.

DR BRIAN RUSSELL (London). I was interested to hear Dr A. Glückmann say that in mice exposed to dimethyl-benzanthracene the absence of hair follicles of a previous exposure to an epilating dose seems to favour the development of sarcomata instead of epitheliomata.

I have recently studied eight cases of fibrosarcoma of the skin (other than dermatofibrosarcoma protuberans) following exposure to ionizing radiation. Three cases followed X ray treatment of scarred hairless patches of lupus vulgaris. Two followed X ray epilation treatment of hirsuties and one of them had also had radon inserted into an epithelioma which developed 6 years before the fibrosarcoma. One developed after X ray treatment for thyrotoxicosis which had been followed by persistent ulceration. One followed radium treatment of an epithelioma which had developed in the scar of a burn and one arose on the tip of the thumb of a technician who had been handling radon. These fibrosarcomata developed from 15–4– years after the exposure to ionizing radiation, an average of 28 years.

WHITTLE. There are a small proportion of tumours with a basal-cell histology which show complete clinical regression so that spontaneous

regression is not confined to squamous cell tumours. I have discussed the point with Professor Mitchell and he agrees that this does occur. Does Dr Foulds agree?

FOULDS. With regard to regression one of the reasons why I introduced the subject of what I call the important carcinomata is because we must approach this subject with an open mind. What I would object to is the statement that if a tumour regressed then it was not a carcinoma by definition. I want to get away from this frustrating or paralysing state of mind, so that we can keep an open mind and find out what really does happen. In the laboratory and clinically there is a good deal yet to learn about the potentialities of tumours and their natural history and I think there is good evidence that regression does occur and quite apart from regression the rate of growth of tumours varies considerably from time to time in a way that the text books often fail to mention.

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RADIATION AND THE SKIN

SOME ASPECTS OF THE MECHANISMS OF THE THERAPEUTIC ACTIONS OF IONIZING RADIATIONS IN MALIGNANT DISEASE

By J S MITCHELL

It is well known that the ionizing radiations produce a wide range of disturbances of biological processes. The majority of these are deleterious. In the radiotherapy of malignant disease, valuable use has been made of these essentially deleterious effects by the application of ionizing radiations under suitable conditions. At the present time, X rays and γ rays, mainly those of radium, are still the only agents, other than surgery which have produced permanent healing of certain types of malignant tumours in substantial numbers of patients. The first cases of cancer healed by X ray therapy were of squamous-celled carcinoma of the skin and were treated in 1899 by Stenbeck & Sjögren in Stockholm. It is often said that radium treatment began as a result of the burn received by Becquerel in 1901 after he had carried a radium tube in his waistcoat pocket for fourteen days (Becquerel & Curie, 1901)

The numerous early radiobiological investigations were directed largely to the development and understanding of radiotherapy Much of the earlier work was forgotten, especially if the significance of the findings was not understood at the time. A striking example of this is the observation of chromosome breakage by ionizing radiations. This was described by Perthes in 1904 and studied in some detail by Paula Hertwig in 1911 However its importance was not realized until in 1927 H. J Muller (1927 1928) made the fundamental discovery that in *Drosophila*, X rays were able to induce mutations indistinguishable from those which appeared spontaneously Shortly afterwards Muller & Altenburg (1930) found that X rays also induced structural changes in the chromosomes, in particular translocations, similar to those occurring naturally About the same time Stadler (1928) showed that X rays and the γ rays of radium produce gene mutations and chromosome breakage in plant cells. This remarkable work inspired numerous biological and biophysical investigations but had practically no influence on medical radiobiology until about 1946 and still has not been fully assimilated into medical science.

Clinical radiotherapy is still empirical. Its development from a fundamental point of view has reached an impasse. It is essential at the present time to provide a basis for the practical application of radiobiology to radiotherapy

For this purpose, it is important to try to form a coherent quantitative picture of the mechanisms by which the measured doses of X- and γ-radiations act upon the cells of a malignant tumour and the adjacent and interwoven normal cells and tissues, and upon the patient as a whole, to produce the selective response which under favourable circumstances results in the permanent healing of the tumour and the cure of the patient. An even more difficult aspect of the problem is the nature of radio-resistance. It is of great importance to understand why some individual tumours of types that often respond, and some types of tumours, are not curable by radiotherapy. Further, one must hope that knowledge of the details of the mechanisms of the therapeutic actions of X- and γ-radiations on human malignant tumours will help to provide a rational basis for the chemotherapy of cancer.

This is a singularly difficult field of biological and medical inquiry. It is clear that collaboration between investigators trained in a wide range of scientific disciplines is essential for progress. The diversity of the evidence concerning the various aspects of the subject is characteristic of biological problems in general. Moreover, it is important to emphasize that the dynamic pathological system under consideration is a very unusual one from the biological point of view.

It is necessary to analyse the clinical observations and the results of experimental studies by all the available qualitative and quantitative methods and for the different levels of organization from the macroscopic down to the macro-molecular and molecular.

HISTOLOGICAL OBSERVATIONS

All the evidence suggests that the most important radio-lesion produced by therapeutic doses of radiation is submicroscopic. Even present methods of electron microscopy do not appear to demonstrate it, although they show the occurrence of changes in mitochondria after small doses of radiation (Scherer & Vogell, 1958).

Nevertheless the methods of classical histology are still fruitful in providing information at the cellular level concerning the mechanisms of the therapeutic actions of radiations. The radiosensitivity of proliferating cells has long been recognized. Microscopic studies have shown that degeneration of radiosensitive cells after therapeutic doses of radiation occurs by one of two general mechanisms.

(1) *The action on proliferating cells* that is demonstrable microscopically in terms of mitotic inhibition and its effects and sequelae, including cell degeneration, continued cell enlargement and probably induced differen-

tation, with recovery processes later if the cells have not been damaged too severely

A temporary oedema of cells and tissues a few hours after therapeutic doses of radiation has often been observed clinically and recognized by histological methods as cell oedema and vacuolization It is of practical importance in the treatment of malignant tumours surprisingly infrequently

(2) *The process of acute cytolysis* in which certain highly radiosensitive cells break down and degenerate after irradiation without the intervention of mitosis. Among normal cells showing acute cytolysis after small doses of radiation, the best known are the small lymphocyte, spermatogonia of late type A, intermediate type and early type B primordial oocytes, the basal cells of the crypts of Lieberkühn and the embryonic neuroblast. Similar behaviour occurs in *some* of the tumour cells in basal-cell carcinoma of the skin. The small lymphocyte is not a mitotic cell but is the most radiosensitive somatic cell. The basis for the radiosensitivity of the small lymphocyte is not understood and presents one of the problems of radiobiology

The general correlation of radiosensitivity with mitotic activity and lack of cell differentiation in a proliferating tissue or malignant tumour has long been recognized. It was expressed in the so-called law of Bergonié & Tribondeau (1906) However this law although a general guide, is not true in detail and in most highly radiosensitive tissues there are primitive cells which are radio-resistant and from which the tissue regenerates if it survives to do so (see Tullis, 1949). The mechanism (1) involving mitotic inhibition, cell degeneration, and in some cases cytoplasmic differentiation is the most important process involved in the retrogression of the majority of the radio-responsive tumours treated by radiotherapy including for example, squamous-celled carcinoma of the skin.

It is important to distinguish between *temporary effects with recovery* such as temporary mitotic inhibition after small doses of radiation, and *permanent effects* In the therapeutic action on malignant tumours, it appears that the moderately high doses of radiation necessary for healing produce irreparable damage to the molecular structure of the nuclei together with permanent inhibition of mitosis, which as a rule is only complete after a number of cell divisions

There is some evidence for the existence of two types of temporary mitotic inhibition induced by X-rays. In the highly radiosensitive neuroblasts of the embryo grasshopper 50 per cent mitotic inhibition is produced by the small dose of 4 r (see Shaw 1958). X ray-induced mitotic inhibition in the dose range below 32 r under aerobic conditions and below 64 r under anoxic conditions appears to behave differently from that at higher doses,

where there is dose rate dependence and a dose threshold, in addition to the prolonged inhibition.

Interference with the mechanism of mitosis is a sensitive index of radiation injury in somatic cells. In general terms it appears that primary damage is produced in a high proportion of the cells of proliferating tissues at the time of irradiation but that the damage is not visible until the cells divide. The action of radiation on the cells during interphase appears to be an important factor in the damage to cells associated with permanent healing of the tumour

For practical purposes, as a first approximation, one can separate the biological effects on human tissues of single localized doses above and below the level of about 750–1000 r of 250 kV X rays. In relation to the minimum tumour dose of approximately 2000 r of this radiation necessary to produce permanent healing of a small squamous carcinoma of the human skin, this threshold level of dose is about 40–50 per cent. *For the usual and more effective fractionated radiotherapy with overall time of treatment between 8 days and 6 weeks, the threshold appears to be somewhat higher in the region of 50–60 per cent of the minimum tumour dose.*

At doses above this threshold level, the effects of radiation upon capillary endothelium and upon collagen, including the formation of collagen, and delayed degenerative effects upon the walls of the blood vessels become important. These delayed pathological effects involving blood vessels are of particular clinical importance in sites where the changes are deleterious, as in the kidneys, the heart, and the central nervous system including the retina.

Selective effects of radiation on different types of cells are important in considering the histological effects of radiation on normal tissues and malignant tumours. The mechanism by which malignant tumours retrogress after therapeutic irradiation is complex. The histological studies suggest that in radio-curable tumours selectively greater injury is inflicted by the radiation upon the tumour cells than upon the cells of the adjacent and interwoven normal tissues. *The damaged tumour cells appear to be prevented from recovery by the normal cells of the so-called stroma reaction.*

For many years, clinicians have been convinced that the resistance mechanisms of the surrounding and interwoven normal cells and tissues are nearly as important in radiological cure as the degeneration of the tumour cells themselves resulting from irradiation In 1928 Forssell wrote The wider our experience becomes the more do we realise the part which the general resistive power of the individual plays for radiological healing Subsequent experience confirms the truth and importance of this statement. However much further work is necessary to study its scientific basis.

Permanent retrogression of a malignant tumour after radiotherapy is usually associated, with and appears to require, a satisfactory stroma response. In the absence of the stroma reaction, many of the tumour cells recover from the initial radiation injury and grow again. This has been emphasized since the experimental work of Cramer (1932) and of Ludford (1932). However most of the animal experiments over many years have been carried out with transplanted tumours. This method can give fallacious results on account of the reaction of the host on the immunogenetically foreign tumour transplants often transplanted tumours can be cured more easily than their spontaneous counterparts (see, for example, Révész, 1958). With regard to the stroma reaction, it has been found that local pre irradiation of the implantation site resulted in growth inhibition of a small inoculum of viable tumour cells (cf. Vermund, Stenstrom, Messer & Johnson, 1956). Révész showed that this inhibition could be partially counteracted by the presence of large numbers of tumour cells which had been heavily irradiated.

APPLICATIONS OF CYTOLOGICAL AND GENETICAL METHODS

The problem of whether or not the production of chromosome structural changes is the most important mechanism by which tumour cells are damaged and killed in radiotherapy is still under discussion. The most relevant processes involved are the loss of chromosome parts following single breaks of chromosomes and the production of inviable chromosome aberrations as a result of asymmetrical interchanges due to breakage of chromosomes by radiation and subsequent reunion in abnormal ways. There is considerable quantitative evidence that in plant materials the induction of chromosome structural changes can explain the lethal effects of ionizing radiations on cells (Sax & Brumfield, 1943 Gray & Scholes, 1951).

However it seems to me that the evidence now available shows that this is not the case in the action of therapeutic doses of X and γ radiations upon human malignant tumours. Professor P C. Koller (1958) was able to carry out detailed cytological analyses of the response of three cases of squamous-celled carcinoma after treatment with single doses of X-rays of 2800–3000 r He found that cell division was suppressed for 6 days and that not more than about 75 per cent of the dividing cells had chromosome injuries microscopically visible at 8 days after treatment. By analysing serial biopsy specimens taken from these tumours, it was ascertained that on the average 8 days after treatment was the time when the number of cells injured by the dose of 2800–3000 r was at its maximum and that after

this time the number of cells with microscopically detectable chromosome injuries decreased rapidly Nevertheless the tumours subsequently disappeared and remained healed.

The role of factors other than the induction of microscopically visible chromosome aberrations in the production of lethal effects on cells is supported directly and indirectly by a considerable range of experimental evidence.

It appears that by increasing the refinement of the methods of study of chromosome injury it becomes possible to demonstrate progressively smaller regions of damage. *In this paper it is suggested that the basis for* the lethal action of therapeutic doses of ionizing radiations upon malignant tumours *is damage to the macromolecular structure of the cell nuclei* It is envisaged that microscopically visible chromosome breakage is an extreme manifestation of this damage to the macromolecular architecture.

SOME PHYSICAL AND PHYSICO CHEMICAL CONSIDERATIONS

In studies of the induction of gene mutations and the effects of radiations upon enzymes and viruses, a large body of knowledge has developed on both the experimental and theoretical aspects of target theory The simplest type of target theory deals with the case of the single-hit type of biological action (Lea, 1946). Here it can be shown that a sensitive volume *in the cell is inactivated or changed by the occurrence within it or very* near to it, of a single elementary physical process of energy absorption, meaning one ionization or more probably a single ion cluster This type of theory has been useful as an analytical tool in explaining a number of quantitative data concerning the production of gene mutations in *Drosophila* by different types of radiations and the inactivation of various enzymes, macromolecules, phages and smaller viruses (see, for example Pollard, 1953). *However the scope of such a theory is limited.*

Many attempts have been made over the years to develop more elaborate forms of target theory The multi-hit target theory is of two main types dealing with the cases in which either a number of hits must be scored in one single target (Dessauer 1922 Blau & Altenburger 1922 Crowther 1924 1926, 1927 Holweck, 1928 Glocker 1932 Timoféef Ressovsky & Zimmer 1947) or at least one hit must be scored in each of a number of *sensitive targets in an organism in order to inactivate it* (Atwood & Norman, 1949).

In recent years this latter type of theory has had considerable success in interpreting quantitatively the inactivation by radiation of yeasts (for

example, Zirkle & Tobias, 1953 Lucke & Sarachek, 1953) and of human tumour cells *in vitro* (Puck & Marcus, 1956). In these latter elegant experiments, the effects of X rays have been studied quantitatively in single cells derived from a human carcinoma of the uterine cervix (HeLa) under conditions such that 100 per cent of the unirradiated cells reproduce in isolation to form a macroscopic colony Puck & Marcus showed that the survival of single cells, defined as the ability to form a macroscopic colony within 15 days, yields a typical two-hit curve. The results treated by means of the theory of Atwood & Norman (1949) can be shown to fit the relation

$$S = 1-(1-e^{-D/96})^{2.1}$$

for the survival S of the reproductive capacity of the tumour cells as a function of the X ray dose D in r The dose for 37 per cent survival for an individual sensitive site in the cell is 96 r The observed value of the intercept of approximately 2 is taken to indicate diploidy At least one hit must be scored in each of two targets. The authors suggest that the lethal effect may be due to a radiation induced genetic defect and that the locus of action may be chromosomal. Simple calculations on the basis of these data show that the two sensitive targets have a mean diameter of about 0·32 μ and a mean molecular weight of about 1×10^{10} It is not impossible that the targets are nucleoli Much further work is required in this field and it is suggested that caution is required in the detailed interpretation of the results.

The relation between dose and effect for different biological processes is of vital importance to the understanding of the effects of irradiation in radiotherapy from a practical point of view Recovery processes play a much greater part in radiotherapy than might be anticipated. The concept based on different rates of recovery of tumour cells and normal cells from the injuries produced by radiation has greatly influenced radiotherapeutic techniques in the past, and still does so. This concept involves the assumption of a slower rate of recovery of the tumour cells than of the adjacent normal cells and is almost certainly an over-simplification which may not be valid even as a first approximation.

The literature contains many experiments and calculations dealing with the problems of direct and indirect types of radiation action upon the cells. The direct type of action is associated with the ionization processes occurring within the sensitive structures. The indirect types of mechanism are based on the picture that the ionization processes produce changes in the media around the structure affected, for example, highly reactive chemical radicals, especially H and OH together with the molecules H_2 and H_2O_2, and in the presence of molecular oxygen, the radical HO_2, in the water of the cell

it must be assumed that the reactive intermediates diffuse and produce the observed biological changes in the sensitive structures. It is of interest that the free radicals produced chemically from H_2O_2 in presence of ferric ions, but not H_2O_2 itself have much the same effect as X rays in the production of chromosome bridges and fragments in barley seeds (Phillips, 1956). Further it was shown by Kimball, Hearon & Gaither (1955) that H_2O_2 is not a mutagen in *Paramecium*. The possible role of organic peroxides must also be considered, and has been examined in detail in a recent book (Latarjet *et al.* 1958). The problem is a very difficult one and no final assessment is possible at the present time. However there appears to be no conclusive evidence that organic peroxides play an important part in the action of ionizing radiations on mammalian tissues.

Important physical evidence for the production of long lived organic free radicals in irradiated tissues has been provided by the studies of electron paramagnetic resonance spectra by Zimmer Ehrenberg & Ehrenberg (1957) in barley embryos, more magnetic centres were produced after irradiation in air than in nitrogen, and some of the centres persist for times of 2 days and longer.

In recent years, studies of the effects of radiations on polymers and particularly the demonstration of protection against radiation and of oxygen effects in solid polymers has made the distinction between direct and indirect effects of radiation much less sharp than was previously thought (Alexander & Charlesby 1954, 1955). The term indirect type of action should be reserved for fundamental considerations of the mechanism of action of radiations and separated from indirect physiological effects due to diffusible substances.

It seems to be necessary to reconsider the importance of direct effects. It is well known that experiments on the inactivation by radiation of many molecules of biological interest in a dry or heavily protected state have shown a high correlation between the target size and the molecular weight (Lea, 1946 Pollard, 1955 Guild, 1958). In considering the evidence it seems clear that in all these discussions of the mechanism of the therapeutic action of ionizing radiations, some essential physical process has been ignored. It seems likely that under some conditions in the nucleus (and probably also in the cytoplasmic organelles) of the living cell the highly oriented macromolecular systems approximate in their physical properties to the solid state. The possibility that chloroplasts may be semi-conductors was first suggested by Szent Györgyi (1941) and the recent experiments of Commoner Heise & Townsend (1956) and Arnold & Sherwood (1957) provide evidence for this. Studies of paramagnetic resonance spectra provide evidence for electron transfer between molecules in solids irradiated

with X rays and show that such a mechanism could account for the protection of catalase from radiation inactivation by glutathione (Norman & Ginoza, 1958). It is suggested that solid-state physics, with especial reference to semi-conductors and organic crystals, is immediately relevant to the problems of radiobiology under discussion.

THE CELLULAR METABOLIC DISTURBANCES PRODUCED BY IONIZING RADIATIONS

Until about 1935 it was generally accepted that doses of radiation of the order of magnitude used in radiotherapy had little effect on the biochemical processes occurring in living cells. This conclusion was based mainly on studies of respiration and glycolysis for which as a rule changes are only demonstrable after much larger doses, in the region 10 000–100,000 r or even higher. A useful example of the work at that time is the investigation by Hubert (1929) of the effects of irradiation of the 5-day chick embryo. A dose of 2000 r of X rays completely stopped the growth of the embryo and led to its death, but had no detectable effect on respiration or glycolysis. A dose of 12,000 r caused only a 15 per cent decrease of respiration but reduced glycolysis by 45 per cent. This type of result has been abundantly confirmed by subsequent work. One exception of particular interest among the early studies was the discovery by Benjamin & Sluka (1908) and independently by Hektoen (1915) of the reduction of the primary antibody response by single sub-lethal and lethal doses of X rays in rabbits. It seems that these important observations were forgotten and attracted little interest until recently.

It is now generally accepted that therapeutic doses of ionizing radiations inhibit the synthesis of deoxyribonucleic acid (DNA) and produce a disturbance of cellular nucleic acid metabolism in proliferating cells. These processes were demonstrated first by Euler & Hevesy (1942), using radiophosphorus, ^{32}P as a tracer and by myself (1940, 1942*a*, *b*, *c*, 1943) by the use of ultra violet photomicrography combined with photographic photometry. However, as often happens in scientific investigations, this relatively simple picture has proved to be very incomplete. Subsequent experiments, especially those with low doses of radiation, have revealed the great complexity of the disturbance of cellular nucleic acid metabolism and the associated far reaching influences of this disturbance on the biochemical behaviour of the cell.

INHIBITION OF THE BIOSYNTHESIS OF DEOXYRIBONUCLEIC ACID (DNA)

It appears to be generally accepted that the relatively high doses of radiation necessary to achieve permanent healing of malignant tumours produce inhibition of the formation of new DNA. There are very wide differences between tissues in the effects of irradiation upon the DNA metabolism (cf. Kelly, Hirsch, Beach & Payne, 1955).

For some years it has been recognized that a problem is raised by the finding that these and larger doses of radiation reduce the uptake into DNA of precursors with radioactive labelling only to about 50 per cent under conditions in which there is no destruction of cells. This inhibition of incorporation of radioactive precursors into DNA reaches its maximum value in the region of about 50 per cent within a few minutes after irradiation. For example, Ord & Stocken (1956, 1957) have shown that a dose of 1000 r of X rays delivered in 3 min. 40 sec. reduces the incorporation of ^{32}P into the DNA of rat thymus to 50 per cent at 3 min. after the end of irradiation. There is little change in the degree of incorporation during the next 2 hr. By 24 hr., when profound histological changes have occurred, incorporation has practically ceased.

Recently, the relationship between the reduction of incorporation of precursors with radioactive labelling into DNA and the dose of radiation has been determined both for a highly radiosensitive tissue, the rat thymus (Ord & Stocken, 1958) and for the less radiosensitive human bone marrow cells cultured *in vitro* (Lajtha, Oliver, Berry & Noyes, 1958). In both cases there is a rapid reduction of incorporation of precursor into DNA with small doses, the effect increasing with increasing dose in this region down to about 50 per cent of the control value; then for higher doses there is a further reduction of incorporation which increases much more slowly with increasing dose of radiation. For incorporation of ^{32}P into rat thymus DNA at 2 hr. after exposure to X rays, approximately 50 per cent inhibition was reached after 200 r; at higher doses the inhibition increased slowly, the incorporation having the value of about 40 per cent of that of the controls after 400 r and 25 per cent after 1600 r.

For incorporation of ^{14}C labelled formate into the DNA of the cultured human bone marrow cells at 4 hr. after irradiation, a dose of 2000 rads. of X rays reduced the synthesis to about 50 per cent of the normal rate. The relation between the depression of the rate of synthesis of DNA and the dose of X radiation was analysed in terms of two exponential components: for the radiosensitive component, the dose corresponding to 37 per cent ($1/e$) residual incorporation was found to be about 500 rads, while for the

radio-resistant component, the dose similarly defined was 13 000 rads. The relative biological efficiency for radon α-particles at doses of 500 and 5000 rads. was found to be about 0·5 These results refer to effects of radiation during the period of synthesis of DNA. It was considered that it was most unlikely that the findings could be attributed to the existence of populations of cells with different radiosensitivities. One must raise the possibility of two alternative metabolic pathways of synthesis of DNA, one radiosensitive and one radio-resistant, or even two types of DNA with turnover of different radiosensitivity (Harbers & Backmann, 1956).

It is important to note that no differential effect of irradiation on the incorporation of different precursors into DNA has been detected with certainty If confirmed, this evidence suggests that irradiation interrupts a mechanism of synthesis in which the components are built into a macromolecule as a single process.

During the amitotic period for some hours after a single moderate dose of X rays—*as studied in the cells of the Ehrlich ascites tumour after* 1250 r (Klein & Forssberg, 1954 Forssberg & Klein, 1954) and in the Yoshida ascites tumour after 1000 r (Gardella & Lichtler 1955)—the formation of new DNA is inhibited and the content of DNA per cell remains substantially unchanged or shows only a very slight increase at the same time, there is more or less normal progressive and roughly proportional increase in the mean cell volume and content of ribonucleic acid (RNA), nitrogen and water

In recent experiments, Caspersson, Klein & Ringertz (1958) have studied the effects of a single dose of 1250 r of X rays *in vivo* on the cells of three lines of Ehrlich ascites tumour of which one was diploid and the other two tetraploid. The irradiation caused a considerable increase in the mean dry mass per cell, average cell volume, and extinction per cell at 2650 Å, while the increase in DNA content per cell was much less. It was found that the cells which contained the double DNA content when they were irradiated were unable to synthesize more DNA, but that those cells which have not yet begun or just started their synthesis of DNA are able to reach the double DNA value. For some years, it has been suspected that synthesis or exchange of DNA can take place in cells as a metabolic function not connected directly with cell division. For example, in thymus tissue the small lymphocytes are non-mitotic but yet show rapid uptake of labelled precursors into DNA often with about 50 per cent reduction after irradiation, as discussed. It now appears to be accepted that in regenerating rat liver the formation of DNA as indicated by the amount of ^{32}P incorporated into the DNA is approximately equal to the increase in DNA P calculated from growth. At Cambridge, using ^{14}C labelled formate or glycine,

already present in the proliferating part of the tumour. In a typical example which I studied for the nuclei of the tumour cells, the mean DNA content per interphase nucleus was found by ultra-violet photomicrographic absorption measurements to be $7 \cdot 8 \times 10^{-12}$ g. Hence there are approximately $1 \cdot 44 \times 10^{10}$ monomeric deoxyribonucleotides of average molecular weight 327 per nucleus. A dose of 2250 r of γ radiation corresponds to the formation within each nucleus, of average volume $169 \mu^3$, of $2250 \times 169 \times 1 \cdot 79 = 6 \cdot 81 \times 10^5$ ion pairs. Hence the ionic efficiency, which is the number of monomeric deoxyribonucleotides whose formation (or duplication) is inhibited per ion pair, is $2 \cdot 1 \times 10^4$. Such a value would correspond to inhibition of formation of one polymeric molecule of DNA of molecular weight $6 \cdot 9 \times 10^6$. This value is essentially identical with the values of about 6×10^6 obtained in a number of measurements of the weight of DNA molecules extracted from various biological materials. Recent measurements on a number of carefully prepared samples of DNA from calf thymus (Rice & Doty, 1957; Doty, 1957) indicate that the average molecular weight is $8 \pm 1 \cdot 5$ million.

Such good agreement is almost certainly fortuitous. The experimental method involves a number of difficulties, and the method of calculation is crude, though the numerical values used appear to be typical. However, these considerations suggest that one ion pair inactivates and stops the duplication of one molecule of DNA (Mitchell, 1956).

The possibility of a direct action mechanism involving DNA or probably the deoxyribonucleoprotein (DNP) system must be raised. From this point of view, these calculations show that the minimum tumour dose necessary to produce permanent healing of a typical small squamous-celled carcinoma of the human skin is such that the mean number of ion pairs per interphase nucleus is equal almost exactly to the mean number of molecules of DNA of molecular weight, 7 millions per interphase nucleus. Although this one-to-one ratio is probably fortuitous, it is likely that the result obtained indicates that there is some meaningful relationship between the number of DNA molecules per nucleus and the number of ion pairs required to produce permanent suppression of mitosis and ultimate death of the cell.

The DNA molecules are envisaged as an intrinsic part of the deoxyribonucleo-protein molecular system. (It may be mentioned that for a nucleoprotein prepared from calf thymus, Doty (1957) found the molecular weight by the method of light scattering to be 19 ± 5 million.) It must be considered as a possibility that only a fraction of the total number of DNA or deoxyribonucleoprotein molecules needs to be inactivated in order to inflict irrecoverable damage upon the nucleus. If the ionic efficiency of inactivation were less than unity by a fraction which fortuitously had a

similar numerical value, *for example, one third, the one-to-one ratio would* still be obtained.

It is suggested that the therapeutic effect is based on the production of a *macromolecular lesion* which involves the deoxyribonucleoprotein (DNP) system. It appears that damage anywhere in the macromolecule stops self-duplication. It is tempting to speculate that there is a mechanism of separation involved in the duplication of DNA or DNP which may be likened to the opening of a zipper and that this can be blocked by the changes produced by one pair occurring anywhere in the sensitive region throughout the whole extent of the duplicating system.

It is suggested that although DNA is not as radio-resistant as the changes in viscosity appeared to indicate, it does *not* show sufficiently high radiosensitivity *in vitro* to make it plausible that changes induced in DNA itself provide the basis for the radiosensitivity of living cells.

Moreover the electric birefringence studies of DNA extracted from *E. coli* after irradiation with a dose of 10,000 r show that the DNA *in vivo* in the bacterial cell is very well protected no changes could be detected (Norman & Field, 1957) No changes have been found in the physical properties of DNA isolated from mammalian cells after irradiation *in vivo* with a dose of 1000 r (Butler 1956 Ord & Stocken, 1957).

On the other hand, there appears to be very substantial evidence that undenatured preparations of deoxyribonucleoprotein (DNP) are highly radiosensitive *in vitro* (Anderson, 1954 Fisher see Hollaender 1956 see also Bernstein & Mazia, 1953*a*, *b* Kaufmann, McDonald & Bernstein, 1955 Cole & Ellis, 1955)

DISCUSSIONS AND CONCLUSIONS

(1) *General aspects*

A number of types of mechanisms of action have been considered in some detail. It must be concluded that many of these constitute facets of the present problem but are not the most important components of the therapeutic action, however relevant they may be for other biological systems. It seems to me that, for example, the evidence now available suggests that the production of microscopically visible chromosome aberrations *per se* cannot explain the action of therapeutic doses of X and γ-radiations upon human malignant tumours. Again in this connection, the production of gene mutations seems to be of little importance. Evidence is accumulating to show that the process of inhibition of synthesis of DNA by radiation is probably not in itself the limiting step in the therapeutic action, although almost certainly closely related to it. Moreover the inter

pretation of radiobiological actions in terms of indirect radiochemical mechanisms appears to me to have reached an impasse. It is difficult to believe that radiobiology can conflict with radiation chemistry but it appears to me that present knowledge of radiation chemistry is quite inadequate to deal with the relevant radiobiological problems. I think it is necessary to reconsider the importance of direct action mechanisms, while at the same time appreciating that indirect types of action play some part.

(2) *The macromolecular lesion of deoxyribonucleoprotein*

In this paper it is suggested that the basis for the lethal action of therapeutic doses of X and γ-radiations upon malignant tumours is selective damage to the macromolecular structure of the cell nuclei. The most important damage appears to be a lesion of the deoxyribonucleoprotein (DNP). The evidence available leads to the conclusion that the changes induced by X and γ radiations in deoxyribonucleoproteins *in vitro* and *in vivo* can provide a quantitative basis for the therapeutic action. DNA itself is not sufficiently radiosensitive and, moreover in the cell it appears to be very well protected against therapeutic doses of radiation.

The macromolecular lesion of deoxyribonucleoprotein appears to provide a quantitative explanation of the lethal effect of a single therapeutic dose of γ-radiation upon a typical small squamous-celled carcinoma of the human skin. It seems reasonable to consider that such a lesion may result in permanent inhibition of mitosis and inhibition of synthesis or of initiation of synthesis of DNA, with secondary disturbance of a great many of the cellular metabolic processes and ultimate death of the cell.

So far there has been little study of refractory tumours from the point of view of the macromolecular lesion. It seems to be important at the present time to determine the molecular—and probably the macromolecular—basis of radio-curability and radio-resistance.

From a general biological point of view it may be considered that the therapeutic dose of radiation denatures the deoxyribonucleoproteins of the cell nucleus. However the details of the processes involved in the macromolecular lesion are as yet only incompletely understood. The evidence available shows that therapeutic doses of radiation lead to depolymerization or breakdown of the deoxyribonucleoprotein macromolecule without damage to the main chain structure of any substantial proportion of the DNA. It seems plausible to suggest that the most important part of the macromolecular lesion is chain breakage affecting the protein closely associated with DNA in the cell nucleus. Probably some of the breaks in the protein chain form cross-linkages with damaged purine and pyrimidine rings of DNA.

(3) *Participation of enzyme systems in the macromolecular lesion*

It is probable that the complex of deoxyribonucleic acid (DNA) and its associated proteins involves a number of enzyme systems, and that some of these are inactivated in the process of damage to the macromolecular system as a whole. Far too little is known as yet about the biochemical processes occurring within the cell nucleus—in particular for the malignant cells. It is of interest that a number of malignant tumours show low levels of diphosphopyridine nucleotide, DPN content, and in some cases strikingly low levels of DPNH together with low content of TPN + TPNH and of pyridine nucleotide transhydrogenase. The participation of DPNH in the response of tumour cells to therapeutic doses of radiation appears likely from the results of my own work on chemical radiosensitizers, especially tetrasodium 2 methyl 1,4 naphthohydroquinone diphosphate (Synkavit) taken in conjunction with those of Strength & Seibert (1955) on the influence of 2 methyl 1,4-naphthoquinone and some related compounds on the enzymatic oxidation of DPNH. Further it seems that insufficient attention has been paid to the possible roles of the nucleolus, and of RNA in the mechanisms of the radiotherapeutic response.

(4) *Relevance of solid state physics and mechanisms of migration of excitation energy*

It seems likely that in the living cell under some physiological conditions in the nucleus, especially in the chromosomes and nucleoli, and probably also in the cytoplasm in the mitochondria, the highly oriented macromolecular systems approximate in their physical properties to the solid state. It is suggested that solid-state physics, with especial reference to semi-conductors and organic crystals, is immediately relevant to the problems of radiobiology under discussion. The macromolecular lesion of deoxyribonucleoprotein (DNP) in the cell nucleus is a highly efficient process, in that as a first approximation, one ion pair appears to stop the duplication of, and inactivate the biological functions of one macromolecule of DNP containing one molecule of DNA of molecular weight of about 7 million. It is difficult to escape the conclusion that there exists in the cell nucleus some mechanism for the transmission or diffusion of the effects of the absorbed energy of the radiation over distances in the region of 500 Å. It is suggested that this can occur as a physical process in the quasi-solid oriented macromolecular system within the cell nucleus, and mechanisms for the migration of excitation energy must be discussed in this connection (see Franck & Livingston, 1949). The possibility of electron migration must be considered first. However it is necessary to consider the alternative

possibility of an exciton mechanism because considerations of the ultraviolet absorption spectrum of DNA appear to indicate the existence of interaction between the consecutive purine and pyrimidine chromophoric ring systems.

(5) *Interrelations of tumour cells and normal cells in radiotherapy*

The histological studies of human malignant tumours provide evidence that in radio-curable tumours, selectively greater injury is inflicted by the radiation upon the tumour cells than upon the adjacent and interwoven normal cells. Further, there is a substantial body of evidence, mainly for carcinoma of the uterine cervix, which shows that the cytological and biochemical effects of the radiation on the normal cervical and vaginal epithelial cells are greater in a high proportion of those patients in whom the carcinoma proves to be radio-curable than in those in whom the disease is refractory (Graham, 1947, 1958; Graham & Graham, 1955; Cherry & Glücksmann, 1954; Herovici & An, 1958). It seems likely that systemic and physiological factors play a part in the radio-curability of tumours. Many attempts have been made to increase radio-curability by means of radiosensitizers of which the most important is oxygen (see, for example, Gray, Conger, Ebert, Hornsey & Scott, 1953).

Permanent retrogression of malignant tumours after radiotherapy is usually associated with, and appears to require, a satisfactory stroma response. In the absence of the stroma reaction, many of the tumour cells recover from the initial damage inflicted by the radiation and grow again. It is suggested that the stroma response may be chemotactic in origin and due to diffusible substances including metabolites liberated from the damaged tumour cells.

On the other hand, there is experimental evidence that products escaping from tumour cells killed by X radiation can act as nutrients for the surviving tumour cells and stimulate their growth. It is important to investigate the significance of this effect in clinical radiotherapy.

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TESTING PATIENTS FOR PHOTOSENSITIVITY

By L. A. MAGNUS

THE OBJECTS OF DOING PHOTOSENSITIVITY TESTS

(1) *As an aid to establishing diagnosis*

Photosensitivity tests do not supersede other methods of diagnosis. All available evidence, clinical, histological, biochemical, must be taken into consideration.

(2) *As a guide to treatment*

The results of photosensitivity tests may guide us in the choice of light barrier preparations for use on the skin. Obviously the application used should absorb light of the spectrum causing the disease.

(3) *As an investigative procedure*

A great deal has yet to be learnt about the photodermatoses. It is hoped that new techniques may further our knowledge of these diseases, especially in regard to the study of action spectra by which is meant the wavelengths of light causing disease. It may be that such information will enable us to establish the actual identity of the substance initiating photosensitization.

THE INDICATIONS IN THE CLINICAL PICTURE THAT MAKE TESTS FOR PHOTOSENSITIVITY DESIRABLE

(1) *History*

(*a*) There is a clear time relationship between exposure to sunlight and the onset or aggravation of the eruption. The time interval is generally short—a few hours, and rarely longer than a day

(*b*) There is seasonal variation the skin is worse in the spring or summer and better or clear in the winter One must try to differentiate seasonal variation in photodermatoses from seasonal variation in other types of dermatitis, such as contact dermatitis due to plants.

(*c*) Exposed parts of the body are affected primarily

(*d*) Family history may indicate a familial tendency to photosensitivity and help to differentiate the atopic diathesis from photodermatoses.

(*e*) Occupation there are a few reports of photodermatoses caused by indoor occupations such as electric welding artificial lighting has also

been blamed. However most patients with photodermatoses get their disease from sunlight and many are people who work out of doors.

(*f*) Certain chemicals are photosensitizers, for example, tar sulphonamides, chlorpromazine. It is important to find out if patients have come in contact with them.

(2) *Physical examination*

(*a*) The morphology of lesions in the photodermatoses is not diagnostic. More than one type of lesion may be present in one case.

(*b*) The most characteristic feature of the lesions is their distribution to those parts of the body surface that are exposed to sunlight, namely the back of the hands the face, ears, V of neck, etc. Typically the shaded skin under the chin is spared.

(*c*) The possibility of a general disease such as porphyria or pellagra should be borne in mind in photodermatoses.

LIGHT SOURCES

Before considering the techniques used in light testing we must consider light sources. The ideal source of light is polychromatic and has a wide spectrum of high energy in the ultra violet, visible and infra red regions.

(*a*) *Natural sunlight* As photodermatoses are, for practical purposes, always due to sunlight, the sun should be the light source of choice. It is, however seldom used, because it is so unreliable, at least in this country

(*b*) *Mercury arc* This is the most commonly used and the most readily available artificial light source for test purposes. It is excellent for testing in the sunburn spectrum (290–310 mμ). It has, however disadvantages.

Firstly the mercury arc emits a *line* or band spectrum, the characteristics of which vary according to the design of the lamp

Secondly most therapeutic mercury arc lamps, such as found in hospitals, have, for the purpose of light testing, a very poor emission in the longer wavelengths of the ultra-violet spectrum.

Thirdly the mercury arc emits a spectrum containing shorter wavelengths than those contained in sunlight, that is, energy below 280 mμ The amount of this short wave radiation depends on what material the tube is made of If it is quartz or something similar erythema may result from radiation in the 260 mμ region, and give misleading results in tests concerned with the normal sunburn spectrum

The first two disadvantages may be overcome by using a special phosphor coated mercury arc tube The line spectrum is thereby converted into a broad continuous band which may be made to extend into the region of the longer wavelengths of the ultra violet spectrum Unfortunately such

lamps do not seem to be made in this country They are consequently expensive. There are also certain types of very high pressure mercury arc lamps available, such as those made by Philips, which have a more or less continuous spectral emission. These are, however very costly lamps and have a comparatively short running life.

The third drawback to mercury arc lamps in testing for light sensitivity their emission of wavelenths shorter than those occurring in natural sun light, can be overcome by using filters. These will be discussed later

(*c*) *Carbon arc* Its spectral emission is continuous, resembles fairly closely that of sunlight, and is powerful in the longer wavelengths of the ultra violet region. It is therefore more suitable for testing photosensitivity than is the ordinary therapeutic mercury arc. Its chief drawback is that it is often difficult to maintain at an even intensity Single prolonged exposures cannot always be given because the carbon electrodes may have to be renewed after something like 30 min. burning

(*d*) *Xenon arc* This was introduced for clinical investigations by Professor Kimmig and his co-workers of the Hamburg Skin Clinic. The spectral emission is continuous and similar to that of the carbon arc. Xenon arcs of high power (1 or 2 kilowatts) are unfortunately very expensive, especially if the envelope is quartz. The price of the auxiliary electrical equipment for running them is also high.

(*e*) *Tungsten filament lamp* Lamps of this type can only be used in the visible and infra red spectra as their ultra violet emission is poor

TEST PROCEDURES FOR INVESTIGATING PHOTODERMATOSES

(1) *Erythemal responses with polychromatic light*

This can be done in two ways (*a*) by ascertaining the minimal erythemal dose (MED) by giving serial exposures of graded duration (*b*) by comparing the shade of erythema after a standard exposure.

The former method, ascertaining the MED is usually preferred and will be described in detail A shield of some opaque material, containing a number of windows, is placed on the skin area to be tested. The lamp is turned on and the windows are then closed at intervals so that doses of graded duration are given. The skin sites irradiated are then inspected for erythema during the next 24 hr The smallest dose causing definite erythema is the MED and the result obtained compared with tests in normal persons.

The following precautions must be observed if any accuracy is to be obtained

(*a*) The same part of the body surface must be tested on the patient and control subjects. It is better to use a skin area that is not normally exposed to sunlight so as to avoid errors arising from suntan.

(*b*) The same lamp must be used for the patient and control subjects.

(*c*) The field of illumination must be even. The only way to be sure of this is to make measurements over the field with a photo-electric cell and galvanometer

(*d*) The intensity of emission must be steady throughout the exposure and the same in every test. Keeping the lamp at a standard distance from the skin is not enough because day-to-day variations in mains electric supply may also alter the intensity A photo-electric cell should therefore be used.

(*e*) The relative intensity of the emission throughout its spectrum must be constant and identical in each test. If this is not attained, and it is possible that it is not always, it may account for the erratic results sometimes obtained. Ageing of the light source, solarization of the envelope and the presence of dirt (especially finger grease) on a quartz envelope will all contribute to a fall in output of energy in the shorter rather than the longer wavelengths in the ultra violet spectrum. A check on the relative spectral intensity of a light source must be made with a photo-electric cell used with a series of filters of different transmission, or better with a spectrograph.

(*f*) The skin reaction should be read by the same observer and under standard conditions.

In appraising the results the following points must be considered

(*a*) Normal subjects show considerable variation, even in the same subject. Variations have been ascribed to age, sex, skin colour race menstrual cycle, season of the year and other influences. One must be certain, therefore, before deciding that an MED test is abnormal in a patient, that the result is definitely outside the range of variation in the normal

(*b*) Patients with florid skin diseases, in which there is no reason at all to suspect sunlight playing any important part in the aetiology may have *an abnormally low* MED *Here one can only suppose the skin to be in an* irritable state analogous to that in which false positive reactions are obtained with diagnostic patch tests.

(*c*) Some patients with undoubted photodermatoses will have a normal MED In our experience, however they will show abnormality in other kinds of tests.

(2) *The reproduction of lesions of the actual disease with polychromatic light*

Before one attempts to produce lesions, it is best to know the MED A dose of light about five to ten times the MED is then given and may sometimes produce lesions. This test often fails in our experience. Lesions, if they appear usually take several days to develop and in the commoner types of cases, the so-called polymorphic light eruptions, are most usually papular sometimes vesicular In the case of solar urticaria lesions may be produced in a few minutes of an exposure.

It must be remembered that the production of lesions artificially by light does not necessarily prove that a dermatosis is due to light. In lichen planus lesions closely resembling the natural disease can be produced this also occurred in a case of prurigo nodularis that we have recently studied. These effects must be regarded as examples of the Koebner phenomenon.

(3) *Finding the action spectrum in a case of photodermatosis*

From my remarks on light sources, it will be evident that the ordinary medium pressure therapeutic mercury arc is not suitable for investigating action spectra except in the region of the sunburn spectrum (290–310 mμ) A super high pressure mercury arc can be used, but probably the carbon arc and xenon arc are better as both have a more continuous spectrum and a higher output in the long ultra-violet region.

The methods for separating wavebands for testing are (1) with filters (2) with a monochromator Whichever method is used, the technique for investigating photosensitivity is the same, the MED is estimated and attempts are made to produce the actual lesions of the disease. These tests have to be repeated in various parts of spectrum, even in the visible and infra red regions, and from the data obtained an attempt is made to construct an action spectrum

FILTERS

These may be divided into two types, absorption and interference. Absorption filters made of dyed gelatine are available for use only in the visible spectrum, but in fact may transmit ultra-violet light—a fact that is usually not disclosed by the manufacturers descriptions. Absorption filters made of various types of glass and quartz are also available They are of two main varieties, the 'cut off' type and those with a more or less narrow zone of transmission in the ultra violet spectrum, of which the Woods light filter is an example. The best 'cut off' filters come from Germany and the U.S.A. and are therefore expensive but ordinary window glass, plate glass, a microscope slide or even a cover-slip can be

used as a cut-off filter as they transmit little or no ultra violet light of wavelengths shorter than about 310–320 mμ. Patients with photosensitivity will sometimes show an erythema if irradiated strongly through plate glass with a light source such as a carbon arc, whereas control subjects will show no such reaction because the normal sunburn spectrum is absorbed by the filter. It is important to avoid contaminating glass filters with finger grease or serious errors may occur in the results of tests.

Interference filters for use in the ultra violet spectrum have only recently been introduced. Their reliability is yet to be established. Although transmitting much narrower wavebands of light than absorption filters, their total transmission is poor. They are also exceedingly expensive, as they require considerable skill in the manufacture.

As mentioned earlier, filters can be used to ascertain the MED of a patient's skin at various parts of the spectrum, and they can be used to see if one can reproduce lesions. Another method is that used by Dr Wiskemann of the Hamburg Skin Clinic. Here the skin is irradiated through a number of filters (6–8) placed individually on different parts of the back. A single long exposure is then given which is of a dose that will have no effect on normal skin. Knowing the transmission characteristics of the filters, the appearance of erythema under any of them will give an indication of the action spectrum. The information obtained is, of course, approximate, but enough to guide the clinician as to what light barrier preparations are likely to be helpful. This method of testing with a battery of filters all at once has led to a lot of information being collected rapidly and easily. It has been shown thereby that patients with the so-called polymorphic light eruption have an action spectrum that is often quite broad, extending from the sunburn region (about 300 mμ) right up towards the visible range (400–800 mμ), sometimes even including part of the visible spectrum. This method of testing is, however, qualitative only. An example of the type of results obtained is shown in Table 1.

Table 1

To illustrate the method of ascertaining the action spectrum in cases of photodermatosis by using a number of filters placed individually on the skin, usually the back, and irradiating simultaneously. Knowing the transmission of the filters, erythema under any of them gives an indication of the action spectrum. The conditions of the test are arranged so that normal subjects give no reaction.

Filters	Erythema in normal subjects	Erythema in light sensitive patients
None	–	+ +
Transmitting 370–400 mμ	–	± or –
Transmitting 300–400 mμ	–	+ +
Transmitting 320 mμ upwards	–	+ + or +
Transmitting 370 mμ upwards	–	± or –
Transmitting 400 mμ upwards	–	± or –

MONOCHROMATORS

The best way of studying the effect of narrow bands of light on the skin is with a monochromator This is essentially a research instrument, is costly and relatively elaborate, and requires supervision by a physicist and the facilities of a technical optics laboratory behind it for its maintenance. A monochromator breaks up the light spectrum by means of a prism, preferably quartz. It is essentially a spectrograph in which the photographic plate is replaced by the patient's skin. The light source must be very powerful, namely a carbon arc, a xenon arc or a super high pressure mercury arc.

Since the beginning of this year Dr Porter and I have been using a monochromator at the Institute of Dermatology It was designed for us by Professor W D Wright of Imperial College, London. This instrument can be used for studying photosensitivity by finding the MED and by attempting the reproduction of lesions. Its most useful range is from 250–400 mμ. Although we can project the whole ultra-violet light spectrum at once on to the patient's skin, we find that it is better to select a number of narrow wavebands and test each separately This makes the investigation lengthy and requires considerable co-operation from the patient to study one patient may take up to two weeks. The results of the tests may be expressed in the form of a graph, of which an example is shown in Fig 1 obtained from a patient with solar eczema. Here the values of minimal doses for erythema and the approximate ones for papule formation are shown together with average values for the normal MED It will be seen that in this patient erythema can be produced over a wide range in the spectrum with much smaller doses than those required by normal subjects. This abnormality extends almost into the visible spectrum. Not all patients with the common types of photodermatoses show such a comparatively marked and widespread susceptibility to light. In many the erythema curve is normal, the only abnormality being the appearance of a papular response when the dose is three of four times higher than the MED

A patient of interest, that has been studied very recently was a case of solar urticaria. This patient produced a whealing reaction from 280 to 390 mμ and from 490 to 560 mμ. Solar urticaria has been classified into two groups depending on whether the wavelengths of the radiations that precipitate lesions lie above or below 370 mμ. Our patient seems to have the characteristics of both these groups. A similar case was reported by Wiskeman & Wulf (1956).

Another patient, who was of particular interest, was one with porphyria cutanea tarda. In this case an oedematous reaction with ecchymoses was

produced at 400 mμ. This wavelength is at the region of the maximal absorption of porphyrins and strongly suggests that photosensitivity in this patient at least, is due to the presence of these substances in the skin.

From a rather brief experience with this instrument, the advantages in the use of a monochromator seem to be several. Firstly the relative energy output in the spectrum can be accurately measured and controlled. Secondly it seems easier to reproduce actual lesions with monochromatic light than with polychromatic light. The reason for this is not clear. Thirdly a much

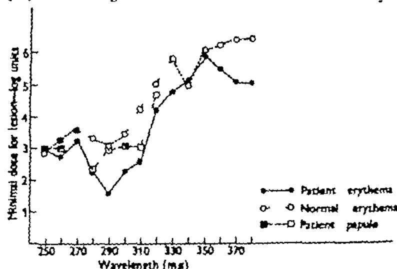


FIG. Action spectrum in case of solar eczema showing values of minimal doses required for erythemal and papular responses. A vesicular response (not shown in graph) was obtained at some wavelengths with dose two or three times that required for the papular response. Average values for normal minimal erythemal dose are shown for comparison. The dose is in logarithmic units namely log_{10} $(t \times I)$, where time (t) is in seconds, relative intensity (I) is in arbitrary units. Intensity measurements were made with Schwarz linear thermopile.

better idea of the action spectrum can be presented. The data are more quantitative. Fourthly it seems that it may be possible in a few cases for us to get an idea of the level in the skin at which the photosensitizing action is initiated, by taking into consideration the transmission properties of the skin for light, with the wavelengths that induce abnormal reactions.

With a monochromator the results of light tests in patients with the polymorphic light eruption seem, so far to be more consistent with the clinical picture. When the history and physical examination of a patient strongly suggest a photodermatosis, it is rare not to find some abnormality. This is not our experience in tests with polychromatic light in our hands they have been disappointing and misleading. The monochromator seems, therefore to be the instrument of choice. It also holds promise of being a powerful weapon for research in this field.

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ELECTRODE STUDIES

By I. A. SILVER and D. B. CATER

Noble metal or glass electrodes inserted into the tissues of experimental animals or human volunteers and patients may give information that is of interest both to the clinician and to the pure scientist. Electrodes of these types can be used to investigate tissue metabolism in three ways

(1) The *potential* of a noble metal electrode in equilibrium with the surrounding tissues will give an indication of the oxidizing or reducing state of the tissue. These electrodes are also sensitive to changes in pH so that such changes must be observed separately on a system that is sensitive only to pH.

(2) The glass electrode responds only to pH. It is unaffected by changes in redox potential and oxygen tension.

(3) If a small negative potential is *applied* to a noble metal electrode in a solution such as tissue fluid the current that flows is proportional to the concentration of oxygen in the solution and hence to the oxygen tension.

Oxygen tension and redox potentials can vary independently of each other and of pH but changes in pH will affect the measurement of redox potentials with metal electrode systems although they will not affect oxygen tension measurements. Variations of redox levels may of course follow changes in oxygen tension.

OXIDATION REDUCTION POTENTIALS AND pH

A platinum or gold electrode inserted into a tissue or a model redox system acts as loose meshwork of electrons and will accept or lose electrons until it is in equilibrium with the electrons in the solution. In highly reducing conditions as when reduced metabolites are present, these will act like hydrogen and donate electrons to the noble metal which will become negative Thus we find that actively metabolizing tissues like brain tumour or mammary gland show a low (relatively negative) redox potential Conversely in subcutaneous tissue which is relatively inactive metabolically compared with its blood supply the conditions tend to be more oxidizing and the electrode will tend to lose electrons and so become more positive (see Cater 1959).

Resting levels and changes of oxidation reduction potentials induced by drugs hormones, toxic agents and radiation in normal and tumour tissue have been followed (Cater Phillips & Silver 1957*a* *b* *c*). Changes of

redox potentials can occur very rapidly and different agents produce characteristic patterns of change in different tissues. However the most striking aspect of tissue redox levels is their constancy and the way in which they return to their normal potentials even after drastic interference by drugs or toxic agents. This indicates a high degree of redox buffering but unlike pH, redox levels can undergo very extensive temporary distortion without death occurring

Glass electrodes for measuring pH *in vivo* should give readings of about 60 mV per pH unit. The design of *in vivo* glass pH electrodes which we have used is based on the capillary electrode of Voegtlin, Kahler & Fitch (1935) (see Silver 1958). They are extremely fragile and are not usually suitable for clinical use although there are some now available commercially

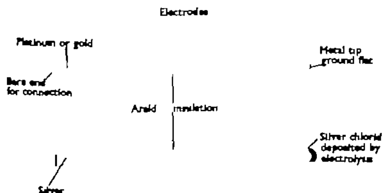


Fig. Noble metal electrode 330 μ diameter and silver/silver choride electrode insulated with Araldite.

for this purpose. The potentials from glass electrodes can be recorded in the same way and with the same apparatus as those from noble metal electrodes (Cater *et al.* 1957*a*) The reference electrode for both pH and redox measurements must be in contact with subcutaneous tissue if reliable information is to be gained, and for this reason a silver/silver chloride indifferent electrode is best for clinical use. The pH of most tissues is very similar thus there is little difference between the pH of dermis, resting muscle or lactating mammary gland but there are of course large variations of pH on the surface of the skin and in the vagina and this may lead to false readings if indifferent electrodes are merely placed in contact with skin instead of subcutaneously (Fig 1).

In contrast to the constancy of pH throughout the body there are considerable variations in redox potential in different organs. Most actively growing tumours, liver and brain have very negative potentials whereas relatively inactive tissues like depot fat, fascia and subcutaneous connective

tissues have more positive potentials. In necrotic areas such as occur in the centre of some rapidly growing tumours redox levels are extremely low that is, the conditions are strongly reducing and the pH is more acid than in living tissue. After the death of an animal the redox levels of all the tissues fall in a way characteristic of the agent causing death A toxic dose of the radiosensitizer Synkavit (tetrasodium-2 methyl 1.4 napthohydroquinone diphosphate) causes very rapid falls of redox, as does carbon monoxide, whereas poisoning with cyanide prevents much change of electrode potentials from the live state for several hours. pH changes appear immediately after death or even before if the animal dies slowly There is usually a rapid increase of acidity of about one pH unit in the first hour after death followed by a much slower increase.

OXYGEN TENSION

The measurement of oxygen tension in tissues using electrodes is simple in principle but very difficult in practice if absolute values are required. A gold or platinum electrode insulated except at the tip is inserted into the tissue to be studied and a silver/silver chloride reference electrode placed subcutaneously A small potential (between 0·5 and 0·8 V) is applied so that the noble metal is the cathode. The amount of current that flows in the system at this voltage depends on the diffusion of oxygen to the surface of the electrode, the area of the electrode surface and the time after the potential is applied to the electrode. The last two factors can be made constant and since the amount of oxygen diffusing in a given fluid is a function of the concentration of dissolved oxygen which is proportional to the oxygen tension, the current can be related directly to the oxygen tension. This is probably true of the observation of changes of oxygen tension in tissues, but unfortunately calibration of electrodes of the simple flush type presents great difficulty as it is impossible to reproduce exactly the complex of cells, fibres and tissue fluid *in vitro* that interfere with the diffusion path of the oxygen *in vivo*

We have made a large number of experiments to try to find a satisfactory method of calibrating electrodes for absolute measurements and feel that we can now measure, approximately absolute oxygen tension levels in tissues. A small compact apparatus has been designed to follow oxygen tension changes in patients, with special reference to the changes in tumours which occur as a result of irradiation. The apparatus (Fig 2) measures the steady state current at four electrodes in a patient, when a constant potential is applied. The d.c. transistor amplifier of high gain and stability feeds the current to a microammeter

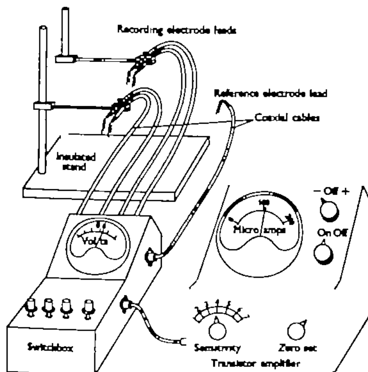


Fig. 2. Apparatus in clinical use for measuring oxygen tension, showing the stand with clamps carrying co-axial leads, the small switch-box and potential source, and the transistor amplifier

INVESTIGATONS WITH ELECTRODES

Redox potential changes have been used extensively to follow the effects of drugs, especially radiosensitizers and radioprotectors, and hormones on normal and abnormal tissues (Fig 3). For example, the radiosensitizer Synkavit has been shown to affect the redox potentials of spontaneous tumours in man and dogs and experimental tumours in rats, in a manner different from its effect on the potentials of normal tissues. It is most important that a radiosensitizer in clinical use should exert a differential effect on tumour and normal cells either by being concentrated in the tumour or by being retained in the tumour after it has disappeared from normal cells. In skin deposits from a mammary carcinoma in a bitch the electrode potentials showed that falls and recovery of redox potentials in the normal skin and mammary gland were very much more rapid than in tumour after injections of Synkavit in therapeutic radiosensitizing doses (Fig 4). Radiotherapy was therefore given over the period in which there was the maximal differential effect of the sensitizer in the normal and abnormal cells.

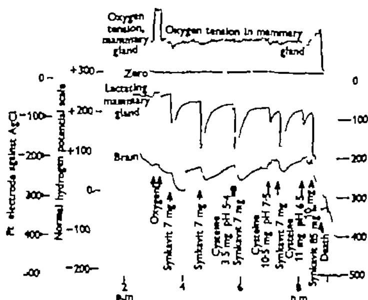


Fig. 3 The effect of Synkavit and cysteine upon oxygen tension and oxidation-reduction potentials in rat brain and lactating mammary gland.

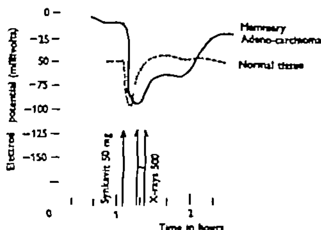


Fig 4. Changes oxidation-reduction potential in skin deposits from mammary carcinoma and adjacent normal tissue of dog fter the intravenous injection of 50 mg Synkavit.

During investigation of possible effects of radiation on redox potentials it was found that as little as 100 r 220 kVp X rays resulted in the production of hydrogen or a hydrogen like radical which caused marked falls in the potentials of occlusive (Pt, Pd and Rh) electrodes but not in non-occlusive gold electrodes.

In dead tissues in a live animal redox potentials are very low and do not alter when oxygen or inert gases are breathed and they are not affected by injection of drugs. This may be useful in assessing the viability of a damaged area of skin or a whole limb

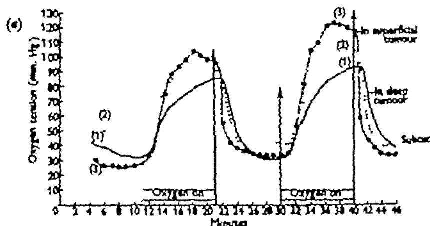
The so-called bio-electric potentials between wounds and skin (Burrows, Iball & Roe, 1942) and between carcinoma of cervix and skin (Langman & Burr 1942) are probably complexes of membrane potentials, salt concentration potentials and pH differences and seem unlikely to have any precise significance since any alteration of skin pH would be likely to cause alteration of the potentials.

OXYGEN TENSION INVESTIGATIONS

Experiments by Davies & Brink (1942) on laboratory animals showed that polarographic methods of measuring oxygen tension could be successfully applied to mammalian tissues. They followed continuous changes in oxygen tension with flush-ended electrodes and found that they could make intermittent readings of absolute oxygen tension with recessed electrodes. Unfortunately recessed electrodes are not suitable for clinical work owing to their fragility if small and to the long intervals that are necessary between readings.

Montgomery & Horwitz (1950) tried to measure absolute oxygen tension in the skin of patients using flush electrodes which were calibrated in dead skin. Owing to their low calibration readings in this system their observations *in vivo* suggest a very high oxygen tension in skin. Penneys (1952) followed changes in skin oxygen tension and compared it with alterations in the arteriolar oxygen tension as measured with a Millikan oximeter

In view of the importance of oxygen as a radiosensitizer (Gray Conger Ebert, Hornsey & Scott, 1953) Cater *et al.* (1957*b*) investigated changes of oxygen tension in tumours of experimental animals in relation to changes of tension in surrounding normal tissues when oxygen was breathed at atmospheric pressure. More recently Cater & Silver (1958) have extended these observations to patients both before and after treatment by radiotherapy (Fig. 5). Observations have also been made of the effect of Synkavit on the oxygen tension in tumours and normal skin and muscle in human patients and experimental animals. Most of the studies in patients have been on epitheliomata or metastatic skin nodules from mammary carcinoma. Despite the dramatic alteration of oxidation reduction potentials, little effect on oxygen tension is caused by injections of Synkavit in therapeutic doses. There is some evidence that in Jensen rat sarcoma Synkavit



(b)

Ag/AgCl

(1) Au. in tumour

4 mm

Ulcer

1.6 mm.

(2) Pt. subcutaneous near tumour

Deep extension

(1) Pt. deep in tumour

Fig. 5. Electrodes inserted into skin carcinoma and the changes in oxygen tension recorded by the electrodes when oxygen was breathed on two occasions by the patient *after* a course of radiotherapy

does affect oxygen tension but this may be an indirect effect mediated through alteration in the respiratory rhythm.

We have found that in oedematous cyanotic epitheliomata there is little or no change of the oxygen tension in response to breathing oxygen through a mask at atmospheric pressure before treatment with X rays, whereas after two or three fractions of the treatment have been given the tumour usually begins to show an increase in the resting oxygen tension and this rises when oxygen is breathed. At least two factors may be involved here increased blood flow due to radiation-induced hyperaemia and damage by X rays to many of the cells in the area which limits or inhibits oxygen uptake. This may play an important part in the successful use of fractionated X ray therapy Unfortunately not all parts of a tumour invariably show a higher oxygen tension after therapy which probably indicates that there are areas of relatively undamaged malignant cells which may recover after the treatment has been completed. Much smaller electrodes than those in common use at the present time will be needed in the search for small groups of cells which may remain at low oxygen tensions throughout treatment. Subcutaneous tissue which has been included in the irradiated field also shows increased resting levels of oxygen tension after radiotherapy but the increase is not usually so great as in the tumour

Some preliminary observations on oxygen tension changes in skin and subcutaneous tissue in guinea pigs have been made with reference to the tuberculin reaction (Cater & Silver 1958) These indicated that there was an early increase in oxygen tension at the site of injection of tuberculin but that when a reaction had developed the oxygen tension in the oedematous area was lowered. Caseating tuberculous nodules exhibited extremely low oxygen tensions which were at the limits of sensitivity of our apparatus.

In small laboratory animals it has been found that oxygen is apparently able to diffuse through the skin after death. Oxygen electrodes in brain, muscle and mammary gland register zero current almost immediately after the death of the animal, whereas those in skin or subcutaneous tissue show a rapid initial fall of current but this does not reach zero until many hours after death when signs of bacterial action become evident. This suggests a steady diffusion of oxygen through intact skin producing an appreciable concentration of oxygen in a tissue which no longer uses oxygen at the normal rate.

It seems probable that use could be made of the technique of measurement of pH redox and oxygen tension in assessing the penetration of substances through intact skin. In particular the problem of how much of a potential poison is likely to be absorbed through the skin in an industrial

process might be elucidated before actual clinical cases occur. Any toxic agents which cause changes in cellular metabolism at the site of absorption could be expected to alter the normal levels of redox potentials or oxygen tension in the area.

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DISCUSSION

Chairman DR C. H WHITTLE (Cambridge)

WHITTLE. What was the actual size of the electrodes used, because of course that does matter in some of the possible applications which I have been thinking about?

MR I. A. SILVER (Cambridge). The standard size for routine work has been 330 mμ diameter but we have used electrodes as small as 10 mμ. At that size they are difficult to handle and give a very small current which sets a limit to their possible uses. So far we have not used the very small ones in patients, but they should be useful in finding parts of tumours which are not well oxygenated. I think the non-oxygenated areas are probably very small and using a large electrode there is a considerable element of luck in finding them.

DR A. JARRETT (London). I should be interested to know whether these electrodes can be used for cutaneous blood flow measurement.

SILVER. Only indirectly with this method. You can certainly use other types of electrodes for measuring cutaneous blood flow

DR H. R. VICKERS (Oxford). I think this technique has a tremendous lot of medical applications. One thing I wanted to ask was whether with the very small currents used one is getting also an electrocardiographic effect and whether that plays any part in the changes measured.

SILVER. No it does not. The time relations are completely different. It was used only in the measurement of redox potentials and in fact it does not affect the measurements.

DR G C. WELLS (London). How does the Synkavit work when used as cited?

SILVER. The phosphate group makes the Synkavit water soluble so that it readily enters the cell and is there hydrolized to the free naphthohydroquinone. There is some evidence that it is selectively taken up by tumour cells but the exact mechanism is still the subject of continued research. The radiosensitizing effect may be due to interaction of Synkavit with SH groups in the cell.

DR A. J E. BARLOW (Huddersfield). In inserting the electrodes surely you would expect to get a certain amount of bleeding Surely this might influence the measurements taken?

SILVER. We usually put these electrodes into tumours through hypodermic needles. The point of the electrode is pushed a little beyond the point of the hypodermic needle. If there is haemorrhage round the point of the

metabolites, but Dr Magnus's results would suggest that the problem is not that simple.

The cutaneous response of the patient with porphyria following irradiation with visible light (4100 Å) is similar in type (livid erythema, oedema) to the skin changes that develop in patients receiving haematoporphyrin and X ray for the treatment of carcinoma. Dr Schwartz, of the University of Minnesota, has observed in these patients marked oedema and erythema when the patients were exposed to sunlight adventitiously on their way to and from the hospital to receive Roentgen therapy

MAGNUS. I am only too aware that our method of measuring the MED is not all one could wish for but it's the best we can do at the moment. Dr Rottier tells me that he is designing a machine which measures the MED by measuring skin temperature. This sounds an excellent idea. Nevertheless we seem to attain a pretty good degree of consistency with our method of measuring U V skin sensitivity

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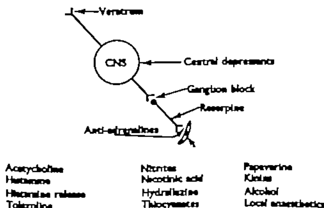
PHARMACOLOGY

PHARMACOLOGY OF VASODILATOR DRUGS WITH SPECIAL REFERENCE TO THE SKIN

By W D M. PATON

INTRODUCTION

The class of vasodilator drugs is not one readily linked together by any very rational pattern and the means of producing a vasodilatation of the skin *selectively* by drugs are extremely limited. I propose to try to arrange the drugs at our disposal in some sort of logical order this may help to make it clear why a selective action on the skin is difficult to achieve. At the end I will turn from discussing the standard vasodilators to one or two other aspects of skin blood-flow which may point to interesting discoveries still to be made about chemical substances and their control of cutaneous vessels.

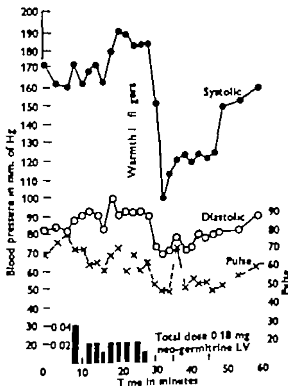


Text-fig. Diagram of sites at which various vasodilator drugs are believed to act.

The scheme I would like to follow is simply to make thumbnail sketches of the different drugs arranged according to their probable site of action, using as a skeleton, the autonomic reflex arc from a site on an afferent nerve, up to the central nervous system, and then out again through the autonomic pathways down to the smooth muscle of cutaneous blood vessels themselves.

VERATRUM ALKALOIDS

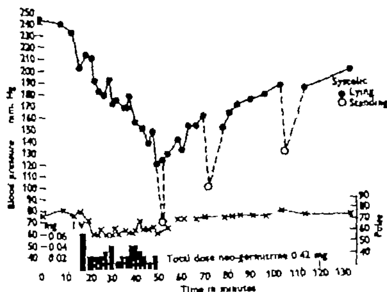
Among the hypotensive agents at our disposal are various derivatives of the veratrum alkaloids. A number of these have been purified and can be used as such but quite often a mixture of partially purified alkaloids is employed, such as Veriloid A main action of these substances is to stimulate afferent endings of the vagus nerve in the region of the heart and



Text-fig 2. Example of abrupt fall of blood-pressure beginning at critical dose in the course of slow injection of neogermitrine. (From Doyle A. E. & Smirk, F H. (1953). *Brit. Heart J* 15, 439.)

the lungs, setting up what is known as the Bezold Jarisch reflex, which on the efferent side slows the heart and relaxes sympathetic tone. There is some evidence that veratrum may also excite afferent endings somewhere in the cranial circulation, since depressor responses have been seen after vagotomy and after injection of veratrum into an isolated cranial circulation. As a result of this reflex a patient receiving a veratrum preparation shows a fall in blood pressure and a slowing of the pulse. The hypotension still occurs after atropine showing that bradycardia is not essential to it. The compounds have been extensively tried in hypertension with some degree

of success. It was believed that a fall in blood pressure could be achieved in this way without associated postural hypotension but it appears that if a substantial fall is produced, then sensitivity to posture is quite severe. The main difficulty in use is that the dose required to produce a useful hypotension or peripheral vasodilatation is very close to the dose which makes the patient nauseated and perhaps vomit. This may in fact be a common feature to drugs which work in this way digitalis, for instance, is known to produce a bradycardia of central origin, and the ability to cause



Text-fig. 2. Prolonged blood-pressure fall persisting after intravenous neogermitrine with demonstration of postural hypotension. (From Doyle, A. E. & Smirk, F. H. (1955). *Brit. Heart J.* 17, 439.)

nausea and vomiting is one of the signs of digitalis intoxication. It is not unreasonable to suspect that wherever afferent excitation of this type occurs, one is liable to produce a sensation of nausea, and that this symptom is part and parcel of the reflex produced by drugs working in this way. A further feature of these drugs, as of many is that tolerance develops to their vasodilator action. For such reasons the veratrum alkaloids are probably of limited use in attempts to promote skin circulation.

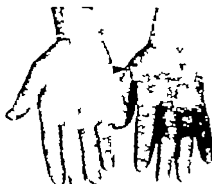
CENTRAL DEPRESSANTS

It is well established that any measure which depresses central nervous function sufficiently intensely will relieve sympathetic tone. The most cogent example of course is spinal anaesthesia, after which peripheral

vasodilatation occurs and postural hypotension may be extraordinarily well developed. A similar state, though usually less intense, can be produced by barbiturates and this forms a basis of the seconal or amytal test for the lability of a hypertension. You will recall that during this test a patient is fairly heavily dosed with these barbiturates and the level to which the blood pressure sinks is then determined. It is supposed, and one thinks reasonably that as a result of the central depression, the autonomic activity responsible for the neurogenic component in vascular tone is removed, so that the cardiovascular system is then only under the influence of its own structural characteristics and the action of any circulating hormones. The use of phenobarbitone in hypertension is presumably a gentler version of the same procedure.

Another instance of the effect on the cardio-vascular system of this kind which may be worth attention is the action of morphine. Normally morphine is not discussed in relation to the cardiovascular system very seriously certainly its analgesic, addictive and constipating action, as well as the depressant action on the respiration are of far greater importance. But in an animal receiving a large dose of morphine, one of the striking occurrences is a prolonged and great fall in blood pressure, reducing the animal to a state not unlike that following destruction of the spinal cord. Further it is now known that in a proportion of subjects receiving ordinary therapeutic doses of morphine, a state of postural hypotension develops. Normally this is not seen since patients usually receiving morphine are supine on a couch, trolley or bed. But if they are made to stand up, then the hypotensive effect can be noticed. Anybody who has received morphine will recall the pleasant feeling of warmth accompanying it I am not aware that this has been analysed but one can readily attribute it to a release of sympathetic tone in the skin. I should mention here for clarity's sake that morphine can produce vasodilatation in quite a different way which I shall mention again later—that of histamine release. One can obtain a rough idea of the relative importance of the two processes from experiments Feldberg and I made Text fig 4 shows how morphine in a small dose can lower the blood pressure without producing the typical picture of histamine release and how with a larger dose, the initial part of the response is attributable to histamine liberation. We have then a general means of increasing the skin blood flow by depression of central autonomic activity But it looks unlikely that one could produce any major depression of autonomic tone centrally without producing a substantial depression of a higher nervous function. I am not convinced that there is any means by drugs acting at this site of producing a selective increase in skin blood flow without any other actions on the body One exception to this may be proposed, the

PLATE I

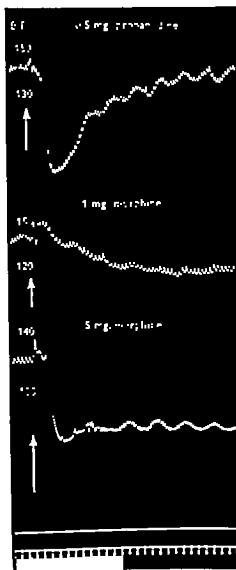


Fig



Fig

For explanation see p. 446



Text-fig. 4. Cat anaesthetized with chloralose. Response to specific histamine-liberating agent, propamidine, compared with that to morphine. The smaller dose of morphine lowers the blood-pressure without significant histamine release. The larger dose produces histamine release in addition, as shown by the abrupt fall in blood-pressure after the characteristic 20 sec latency (From Feldberg & Paton (95). *J Physiol.* 114, 490.)

interesting and confusing compound chlorpromazine. This certainly can reduce the blood pressure, and produces a peripheral vasodilation which includes the skin. Chlorpromazine also has a variety of central actions, notably reduction of vomiting, sedation potentiation of anaesthetics, and a depression of the temperature-regulating centres. But it is also an antagonist to sympathetic motor effects peripherally and although some central depression is difficult to exclude entirely a peripheral sympatholytic action looks to be the main factor in the vasodilatation.

GANGLION BLOCKING AGENTS

With ganglion blocking agents one can attack, specifically the relay stations in the outflow of the autonomic system known as the autonomic ganglia. On the sympathetic side this means one can paralyse the ganglia in the paravertebral sympathetic chains and the prevertebral plexuses. By this means one can produce a substantial reduction of sympathetic tone. Such a method of attack has two great advantages in principle. The first is that ganglion-blocking agents in general do not have central actions. The second is that the effector organ retains its normal responsiveness, so that the effects of ganglion-block can always be countered by drugs like nor-adrenaline or acetylcholine. The absence of central action seems to be complete with agents like hexamethonium and pentolinium, because these substances are quaternary salts this means that, when dissolved in the body fluids, they can only exist in the charged (ionized) form, a condition well known to hinder movement across cell walls. Thus at the blood-brain barrier they are barred from entry into the brain. By the same token they pass the intestinal barrier with difficulty so that oral treatment by such drugs has always offered considerable difficulty The difficulty arises not so much from the fact that they are only poorly absorbed as that absorption is variable thus while normally perhaps about 5 per cent might be absorbed, occasions might arise such as with an intestinal infection or other upset, where the absorption suddenly increased and a gross overdosage could occur

With more recent ganglion blocking agents, however of which mecamylamine is probably at present the best known, substances capable of paralysing ganglionic transmission were developed which are active by mouth and which also can penetrate through other cellular barriers in the body As a result central actions have been described including rather bizarre pictures of tremor mania, and confusional states. Differences from hexamethonium also arise in excretion. Hexamethonium has relatively simple properties it is distributed in the extracellular space it is excreted in the

urine virtually by glomerular filtration no tissue in the body seems to concentrate it selectively All this gives it a duration of action of the order of 4 hr With the non-quaternary salts, however the position is more elaborate. For instance, these drugs are taken up by the cells this may be one of the main reasons why their duration of action is relatively prolonged, for they can enter the cells and then are slowly given off by the cells which took them up As a result, a single dose of mecamylamine may act for as long as 24 hr or even more. Further the excretion by the kidney offers some intriguing features. It is found that if the urine is alkaline, a condition in which mecamylamine would exist more in the uncharged, that is un-ionized, form, then reabsorption takes place quite substantially and excretion is very slow On the other hand, if the urine is acid, when mecamylamine would be predominantly in the ionized form, the absorption is slight and the drug is filtered out relatively rapidly This offers the engaging prospect of being able to control the duration of the action of such a ganglion blocking agent by controlling the pH of the urine. The usefulness of this, however is limited by the fact that the duration of action is also under the control of the reserves of drug locked up in the tissues as a result the extent to which the action can be prolonged or shortened is rather restricted.

So much for some of the properties of these agents in principle. One must mention, too, some of the difficulties in their general exploitation. The main difficulty is that although they only act, if properly used, on a single type of structure, the ganglionic synapse, this nevertheless gives them a width of action often unwanted. Thus the production of a vasodilatation may also entail paralysis of the pupil and ciliary muscle, or constipation or diarrhoea. It is clear that one can increase the blood-flow in the skin of the legs quite strikingly sometimes also in the hands and face but it is difficult to do this without incurring some degree of postural hypotension, or of block of other ganglia. Hexamethonium has been used both to reduce excessive sweating in patients with hyperidrosis and to increase blood flow in patients with vasospastic disease. I think this experience has served more to show that this type of attack is possible, than to establish its usefulness. If it was possible to obtain a drug which only affected those ganglion cells supplying cutaneous vessels then one would have an extremely useful tool. There are, indeed, hints that differential action of this sort is not completely out of the question. When J N Langley was first studying the action of nicotine on ganglia, he described how the different functions of a rabbit s superior cervical ganglion were paralysed in a distinct order indicating that cells controlling the nictitating membrane had a different sensitivity from those, for instance, controlling the blood vessels of the conjunctivae and if one compares the detailed pharmacology of tetraethyl

Table 1 *Summary of the effects of ganglion block in man*

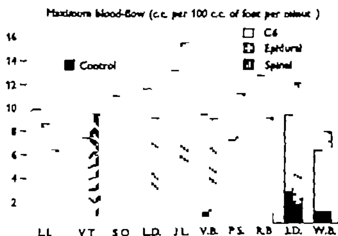
EFFECTS OF HEXAMETHONIUM IN MAN

Sign or symptom	Autonomic pathway blocked	
Flushing of skin and capillary pulsation		to arterioles of skin conjunctivae and muscle
Injection of conjunctivae		
Rise in skin temperature		
Rise in skin blood flow		
Rise in limb blood flow (leg > arm)		
Reduction of cold pressor response		
Postural hypotension sensitivity to lifts	Sympathetic supply (adrenergic)	to arterioles and veins(?) of skin and muscle and viscera
Reduction of bleeding at operation		
Dilatation of retinal vessels		to retinal vessels
Fall in body temperature		to arterioles of skin
Relief of causalgia		to arterioles of affected region
Sensitization to insulin		to adrenal medulla
Dry scalp and dry skin	Sympathetic supply (cholinergic)	to sweat glands
Reduction of reflex sweating		
Paralysis of ocular accommodation and reaction to light	Ciliary ganglion	
Dryness of eyes	Sphenopalatine ganglion	
Dryness of mouth and nose	Otic ganglion and chorda tympani	
Dryness of larynx	Vagal ganglia—in larynx	
Tachycardia	Vagal ganglia—in heart	
Diminished motility of stomach and small intestine	Enteric ganglia	
Constipation	Enteric ganglia	
Ileus	Enteric ganglia	
Diminished secretion of gastric juice: spontaneous, nocturnal, in response to insulin	Enteric ganglia	
Depression of bladder reflexes	Pelvic nerve ganglia	
Impotence	Pelvic nerve ganglia	

ammonium, hexamethonium, pentamethonium and *d*-tubocurarine there are again signs that ganglia vary in their susceptibility and differently with different drugs. But determined efforts to find differential actions of clinical use have failed. Industrial pharmacologists have sought very thoroughly for agents which for instance, would block the sympathetic side and leave unaffected parasympathetic function but out of scores, perhaps hundreds, of compounds no *useful* differential sensitivity has been found.

The usefulness of ganglion block then is rather dependent on one object. In an acute treatment or experiment, where the subject can be under supervision throughout, such drugs provide a very convenient means for relieving sympathetic tone, for a time which the physician can control either by choosing his drug or by choosing his dose For patients with serious hypertension, where the need to lower the blood pressure makes it worth accepting some side actions, ganglion block has an important place. But for less substantial symptomatic therapy such as in vasospastic conditions, one hesitates to suggest blocking ganglia as a means of in-

creasing cutaneous blood flow except for investigative purposes. Such drugs might serve to indicate what profit would be gained by sympathectomy. I believe this has been explored to some extent, with not too fruitful results. This is, in a way, not surprising because the sympathectomy concerned would be local, whereas ganglion block is general and the effect on the limb would depend not only on the local vasodilatation achieved, but also on the effect of vasodilatation elsewhere in the body on the systemic blood pressure.



Text-fig. 5. Chart of the maximum blood flows in the left foot following hexamethonium as compared to lumbar intrathecal or epidural block in ten normal subjects. (From Schnaper et al. (95). J. Clin. Invest. 30, 786–9)

RESERPINE

In recent years quite a new type of drug has appeared. The root of the Indian plant *Rauwolfia serpentina* had for long occupied in folk therapy an important place. Something like four years ago active principles were extracted from it, which were found to produce in animals as their main effect a fall in blood pressure of slow onset and considerable persistence, accompanied by some degree of sedation. At first the action of the main active principle—reserpine—seemed to differ not very much from that of a rather long lasting barbiturate. But then it was found that during treatment with reserpine, the content of hydroxytryptamine in the brain and other tissues was considerably reduced. This gave rise, together with a number of other pharmacological findings, to the idea that both the control of wakefulness and the central control of autonomic reaction were mediated in some way with the aid of hydroxytryptamine in the central nervous system. More recently however Miss Vogt and her colleagues made the important observation that as well as ridding tissues of hydroxy

tryptamine, reserpine also causes a reduction of the nor-adrenaline in nervous tissue. Consequently it can produce an autonomic paralysis which depends, not on a failure of transmission through ganglia, nor on any ordinary style of block at the post-ganglionic synapse, but on a deficiency of transmitter in the post-ganglionic neurone. Further since nor-adrenaline only occurs in sympathetic fibres of the autonomic, the paralysis is restricted to the sympathetic side. Thus we can explain, by a *peripheral* action of reserpine, the hypotension and vasodilatation it brings about, together with such things as the relaxation of the nictitating membrane in dog and cat (which is under sympathetic control), the loss of the ability to dilate the pupil and a relative pupillary constriction as well as features such as stuffiness of the nose and tendency to diarrhoea, due to a now unopposed parasympathetic action. We have, therefore, a means of selectively reducing the activity of the sympathetic nervous system. It is not clear yet how far this reduction of activity goes. A patient on a dose of reserpine which is tolerable in its central effects is certainly not as weak autonomically as one undergoing severe ganglion block. On the other hand, the use of reserpine might be worth considering in some cases where only a modest relaxation of sympathetic tone is required. Reserpine should prove of considerable value for experimental purposes where it is desired to reduce sympathetic activity as selectively as possible. The reason is this if reserpine is given then a depletion of the sympathin content of neurones occurs there follows a period when the reserpine disappears from the body but the deficit of sympathin has not been made good one then has the organism in a state of reduced sympathetic activity yet no drugs are present. At the same time, if it is desired to excite the peripheral effector organ its responsiveness to sympathetic amines is not impaired.

The main complication to the use of reserpine is of course its central action. Normally it has some sedative action which may be beneficial. In some patients, however it may produce a severe depressive even suicidal, state, such that the drug must be discontinued. Sometimes, too it produces a state like Parkinsonism, which disappears a week or so after the drug is stopped. The means by which the central effects are brought about is still obscure. The fact that reserpine can discharge the nor-adrenaline and hydroxytryptamine of the brain, as well as of other tissues, has led to the supposition that these amines are connected with its sedative effect. Recent work on the diffuse neuronal network of the mid-brain known as the reticular formation has also been brought into the picture since there is evidence that the activity of this network controls the wakefulness or otherwise of the brain. Thus it could be suggested, variously that lack of HT or nor adrenaline in the brain, or a flooding of the brain with these

amines when they are released, or a failure to bind them as they are synthesized, distorting the normal pattern of turnover is responsible for the sedation. But it would be out of place to discuss this rather complicated field here. For our main concern, that of how reserpine produces peripheral vasodilatation, it seems that we should attribute this mainly to the peripheral action on adrenergic nerves the persistence of parasympathetic activity seems to weigh against a general central autonomic depression. It may be, of course, that reserpine exercises a specific depressant effect on the central representation of the sympathetic but this is a difficult thing to prove, in the presence of sympathetic paralysis by another cause. For the moment, therefore, we can regard reserpine as possessing an interesting central sedative action (perhaps related to its effects on the local hormones of the mid-brain region), with an ability in the periphery to reduce sympathetic tone by diminishing the content of sympathin in the postganglionic neurone.

ANTAGONISTS TO ADRENALINE

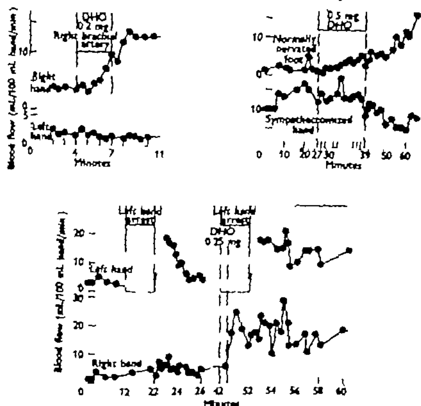
The group of drugs, variously called sympatholytics, adrenolytics, or anti adrenalines, first came to notice with Dale s researches of fifty years ago. He found that in an animal which had received ergot, adrenaline caused a profound fall in blood pressure instead of the customary rise, the so-called adrenaline reversal and that ergot modified the motor effect of adrenaline and sympathetic stimulation in many other ways. Since then pharmacologists have accumulated a good deal more knowledge about this action and acquired many similar drugs, as well as identifying the active principles in ergot. It is believed that they act on the receptor group with which adrenaline or nor-adrenaline combines when it excites smooth muscle, preventing this excitation. The antagonists to adrenaline and nor-adrenaline do not interfere with the sympathetic nerve as such, nor depress the release of nor-adrenaline or adrenaline at the nerve ending, nor interfere with the contractility of the smooth muscle itself But they are what are called competitive antagonists to sympathin at the receptor group and show the same sort of quantitative relationships between dose of antagonist and dose of agonist to produce a given effect as occurs with, say sulphonamides and PABA or atropine and acetylcholine.

One of the most interesting features of these drugs is that it is only certain actions of sympathetic amines which they can antagonize. One can say roughly that those actions of sympathins leading to contraction of smooth muscle are antagonized, but the other sympathetic actions such as the dilatation of blood vessels, relaxation of smooth muscle, stimulation of the heart, central nervous activity or metabolic activity are all antagonized

with much greater difficulty or not at all. In consequence, pressor responses or vasoconstriction of the skin or other vessels are removed, but the tachycardia produced by adrenaline release in the body or other metabolic effects, or the dilatation produced by adrenaline in the muscle bed are resistant to their action (hence the adrenaline reversal). Another feature of their action will, of course, be that parasympathetic actions continue unopposed.

There are three main groups of these agents which deserve separate consideration. First we have the ergot derivatives. The older members of this group ergotoxin and ergotamine, are now less commonly used than substances derived from them chemically the dihydrogenated ergot alkaloids. One of the difficulties of the original alkaloids is that they preface their antagonism to adrenaline by a vigorous sympathomimetic phase. Thus ergotamine has, as its first action, a prolonged vasoconstriction only after this has been passed through does a subsequent fall in blood pressure and a sympatholytic phase begin. The dihydrogenated alkaloids (dihydroergotamine dihydroergocornine, dihydroergocristine, and dihydroergokryptine) lack the sympathomimetic effect and are tolerably good peripheral vasodilators and hypotensive agents, sometimes used in a mixture known as Hydergin. It is interesting however that their action does not seem to be purely peripheral and I would like to go in a little more detail into an experiment in human pharmacology by Barcroft, Konzett & Swan (1951), which indicated that dihydroergocornine (DHO) acts on the central nervous system as well as peripherally to produce vasodilatation, the central action probably being the more important under practical conditions. In Text fig 6 is shown first, the ability of DHO given intra-arterially to a brachial artery to increase hand blood-flow. The next panel shows that it is inactive on a sympathectomized limb indicating that there is no action on the vessels in its own right. All this conforms with its expected sympatholytic activity. There is, however somewhat of a discrepancy between the dose required to produce an effect intra-arterially and the dose which given systemically will produce a good vasodilatation. It is not as active given intra-arterially as one might expect from its systemic effects. Barcroft & Swan (1953) did an ingenious experiment to test for central action, relying on some work which Rothlin had done indicating that after 5 min. the bulk (95 per cent) of the ergot alkaloid is removed from the blood. They first did a control test showing the effect of occlusion of the blood flow to a limb on the blood flow though the hand, a transient hyperaemia occurring when blood flow was restored. After full recovery they then again occluded one arm, and injected the ergot alkaloid intravenously. On the control side it produced the usual vasodilatation. Five minutes after the injection the

circulation to the other arm was restored. One could now presume that it would be exposed to a far smaller probably negligible, dose of the alkaloid and hence, if the alkaloid was acting on structures within the arm, there would be no vasodilatation. They found, on the contrary that after the



Text-fig. 6. Experiment on the mechanism of vasodilatation, in human hand, produced by dihydroergocornine (DHO) Upper left effect of an intra-arterial infusion on normal hand blood flow Upper right effect of an intravenous infusion on blood flow in sympathectomized hand and normal foot. Lower experiment (see text) to show that vasodilatation to DHO still occurs after access of drug to the limb is lessened by temporary arterial occlusion of the limb. (From Barcroft, Konzett & Swan (95). *J. Physiol.* 112, 273)

reactive hyperaemia would have passed off there was a persistent dilatation not much different from that seen in the other hand. This experiment is very hard to reconcile with a purely peripheral action of the alkaloid, but shows a central effect of the drug reducing sympathetic constrictor tone. This is in fact fully compatible with the other pharmacological evidence. It is interesting, too, that among the side effects of the administration of the dihydrogenated ergot alkaloids are central symptoms like nausea and drowsiness. Although then it is probably as well to think of the hydrogenated ergot alkaloids as belonging to the class of anti-adrenalines,

nevertheless one must remember that a central action is probably dominant under ordinary conditions of treatment.

The second class deserving attention can be called the *imidazolines*, because, chemically they contain the imidazole ring, which is incidentally the nucleus of the histamine molecule. In general, like the ergot alkaloids, they produce a reversible antagonism to sympathetic amines. The two most important, tolazoline, also known as Priscol and *phentolamine* also known as Regitine are good enough to be very useful in practice, with a convenient duration of effect, something like one or two hours or sometimes longer The main difficulty with these two drugs is the side effects they produce. Tolazaline is certainly a curious compound. Its relationship to histamine is shown by the vigorous secretion of acid gastric juice which it can elicit. It has a number of smooth muscle stimulant effects including the ability to produce gooseflesh it can evoke chemoreceptor reflexes and it has sympathomimetic actions itself including stimulation of the heart, in addition to a genuine antagonism to adrenaline The most curious feature about it, however is that it appears to have a direct vasodilator action so that the increase in skin blood-flow in the hand which it can cause is greater than with other anti adrenalines in equivalent dose. Phentolamine is a more specific compound, and is probably the anti-adrenaline of choice for investigative purposes. It is free of initial vasoconstrictor action, it is more specific than tolazoline, and it has a duration of action long enough for observations to be made, but not yet so long as to be potentially dangerous. It has been used successfully to antagonize injected sympathetic amines and to help diagnose phaeochromocytoma. In my laboratory it has proved very convenient for antagonizing sympathetic motor effects.

The third group of compounds are those which originated in the work of Nickerson—dibenamine and its relatives. Here we come to a different kind of action the drugs seem to react with the receptor groups in some almost permanent way so that a very long lasting antagonism to adrenaline or nor adrenaline is produced. The drugs are highly specific and the only action they have in ordinary dose is to antagonize motor sympathetic effects, apart from some central stimulant action seen chiefly with intravenous administration and probably unrelated to the drug's main action. Of the group probably dibenzyline also known as dibenyline may well be the most useful. Its duration of action is shorter than that of dibenamine and may be complete in two or three days. It can be given by mouth, and although it is not perfectly absorbed it is rather better and more regularly absorbed than dibenamine and is less irritant. As a drug to be tried in peripheral vascular disease dibenzyline always merits serious consideration.

One last remark should be made on the use of the anti adrenalines, which points, not to the avoidance of their use, but to caution in dosage. Their basic action is to prevent a sympathetic amine from stimulating smooth muscle. If, by any chance, an overdose is given, or an exaggerated hypotension is produced, the question arises, what antagonist you can use to restore the situation. With drugs acting at any point up to the adrenaline receptor drugs such as nor-adrenaline or methylamphetamine are always available to restore the blood pressure. But with a profound fall in blood pressure after a sympatholytic agent one has, for practical purposes, no useful antidote. Posterior pituitary extract can be suggested, since it is not antagonized by anti-adrenalines but it has been found very unreliable as a pressor agent in man, is known to constrict the coronary vessels, and has caused fatalities. Consequently the best that can be done is simply to place the patient head down, so that postural effects are in favour of the blood supply to the head, and allow time to elapse. It is clear that hypotension due to removal of sympathetic tone is not as dangerous as imagined before the days of sympatholytic and ganglion block nevertheless prolonged severe hypotension is very undesirable, requires skilful nursing, and is best avoided.

VASODILATORS

This is such a large and heterogeneous group that they cannot all be discussed fully here. Some of them, histamine and acetylcholine (and its analogues, methacholine, carbachol, furtrethonium) are substances with familiar properties and definite connections with normal bodily function. The vasodilator effects of others, such as the nitrites, nicotinic acid, or papaverine, cannot yet be correlated with any general type of drug action nor with any feature of normal physiology The vasodilator effect of alcohol an action not without clinical usefulness, is probably to be associated with its general depressant action on excitable structures. A few more detailed comments may be necessary about the less familiar drugs. The vasodilatation produced by *histamine liberators* is, of course, very similar to that due to histamine, the histamine concerned coming largely from the mast cells in the skin which are degranulated by the liberator It is sometimes useful to be able to distinguish a direct histaminic action from one due to released histamine this can be quite easily done, qualitatively at least, by reapplying the vasodilator substance to the skin, say twelve hours later If this is done with histamine then the typical response occurs again. But if it depends on histamine release, the vasodilatation is absent or much reduced, simply because the histamine which had been released has not been restored. The fact that the test is possible is an indication of the rather slow turnover time of histamine in the body

CONCLUSION

I am very conscious of having presented rather a mixed collection of therapeutic substances, a sort of pharmacological *bouillabaisse* and it may be rather indigestible. Certainly some of the drugs are rather fishy! Perhaps in conclusion, I should discuss in general what hopes one can entertain for a selective cutaneous vasodilator as a therapeutic weapon. It seems improbable, first, that drugs interfering with the outflow from the central nervous system, whether they act on the afferent, central or efferent path ways, should ever have the required specificity A little more hope attaches, perhaps, to finding substances selectively active on skin vessels, for which tolazoline may be a pointer The difficulty can be by passed of course, by local application—either as ointment or by electrophoresis, and this has been done with acetylcholine and its cousins. But this is not a generally practicable procedure. The vasodilatation mediated by histamine release or by kinins suggests another approach in both cases the localization is achieved, not by a special sensitivity of the skin vessels, but by the localization of the generator of the vasodilator substance—mast cells or glands respectively Here it is the concentration of certain special structures in the skin which determines the site of the vascular responses. One wonders whether the solution to the problem of specific vasodilatation of skin vessels may not ultimately emerge from the specific architecture of the skin itself

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EXPLANATION OF PLATE

PLATE

Quinizarin sweating test on patient with hyperhidrosis. Above, test before injection of pentamethonium. Below same test after injection of pentamethonium. (From Burt & Graham (1950) *Brit Med J* 1 455)

THE MODE OF ACTION OF TRANQUILLIZING DRUGS

By P B BRADLEY

The purpose of this paper is to review some of the investigations which have been carried out with tranquillizer drugs, with the view to elucidating their mode of action on the brain. It will not be possible to cover all the work which has been done with these drugs and I propose to limit my remarks to the types of study with which I and my colleagues in the Department of Experimental Psychiatry in Birmingham have been concerned namely electrophysiological and behavioural studies. In addition, I shall confine my remarks mainly to chlorpromazine, the first drug of this type to be discovered and also the one which has been the most widely investigated. Its pharmacological properties were first described by Courvoisier Fournel, Ducrot, Kolsky & Koetschet in 1953 and many of their early findings have since been confirmed by others. The drug is somewhat remarkable for the wide diversity of its properties, which include among others an anti-emetic action, potentiation of the action of anaesthetics and analgesics, facilitation of hypothermia, hypotensive properties and an antagonism to the effects of adrenaline.

Chlorpromazine is best known, however for its striking psychological effects, which have resulted in the widespread use of this drug in the treatment of psychiatric patients. The first report of its clinical effects by Dealy Deniker & Harl in 1952 has been followed by an enormous volume of literature during the last five years dealing with its therapeutic uses. There is at the present time, however some doubt as to how much of the improvement in the treatment and prognosis of psychiatric disorders is directly due to the use of tranquillizers and how much of it is attributable to other factors, but this is not the time or place to discuss this question.

Chlorpromazine reduces motor activity and in this way calms over-active psychotic patients but without producing generalized depression or clouding of consciousness. These effects are believed to be due to an action on the central nervous system and the reduction in motor activity can be seen in experimental animals when the drug is injected (Bradley & Hance, 1957 Das, Dasgupta & Werner 1954). The changes in locomotor activity are accompanied by changes in the electrical activity of the brain (Terzian, 1952) and early workers who recorded changes in the electrical activity of the cerebral cortex in animals (Longo von Berger & Bovet, 1954 Hiebel,

Bonvallet & Dell, 1954), observed that the generalized cortical response to external stimuli was suppressed following the administration of chlorpromazine. This response, the arousal response, is thought to be mediated by the brain stem reticular activating system, and it was therefore suggested that the drug acted on this region of the brain and depressed the activity of the reticular formation. We have investigated this idea more closely and produced some evidence from experiments with animals which lends support to it, and we have put forward a further hypothesis for the action of this drug.

Our investigations into the action of drugs on the brain have been carried out in four different types of experiment:

(1) Experiments with conscious, unrestrained cat preparations carrying permanently implanted recording electrodes so that the electrical activity of the brain can be recorded simultaneously with observations of behaviour (Bradley & Elkes, 1953). In this way changes in these two variables with the administration of drugs can be observed and correlated.

(2) Acute preparations in which changes in the electrical activity of the brain can be correlated with changes in blood pressure etc. and the effects of lesions on responses to drugs can be examined.

(3) Experiments with specific responses, such as the arousal response.

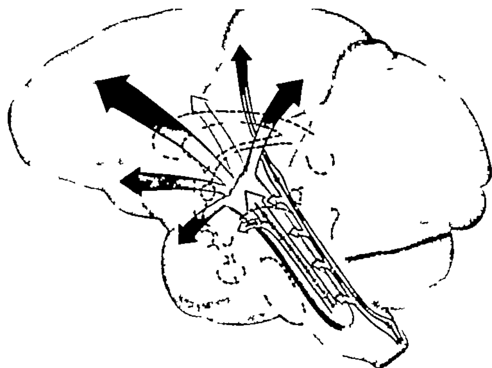
(4) Micro-electrode recordings of the effects of drugs on the activity of single neurones in the brain.

In the conscious, unrestrained animal, the pattern of electrical activity which can be recorded from the cerebral cortex correlates well with the behavioural state of the animal and different patterns can be distinguished according to whether the animal is fully awake and attentive, or drowsy and sleeping (Bradley & Elkes, 1957). The changes produced by a sensory stimulus (arousal response) appear simultaneously in the EEG and behaviour.

The effects of chlorpromazine in these animals were to make them lethargic and indifferent, and at the same time induce an EEG pattern similar to that seen in the early stages of sleep or drowsiness (Bradley & Hance, 1957). The effects of sensory stimuli which previously had caused arousal both in terms of the EEG and behaviour were blocked completely thus confirming the observations of Longo *et al.* on electrical responses alone. The antagonism between the effects of this drug and others such as amphetamine and *d*-lysergic acid diethylamide (LSD 25), together with the results of acute experiments with chlorpromazine, supported the idea that the site of action of this drug is probably at the brain stem level (Bradley & Hance, 1957).

We next examined the effects of this drug in relation to brain stem reticular activating system itself. The ascending influence of the reticular

PLATE I



For explanation see p. 453

formation of the electrical activity of the cerebral cortex was first demonstrated in 1949 by Moruzzi & Magoun, who showed that stimulation of points within this system caused widespread changes in the electrical activity of the cerebral cortex. These changes were similar to those seen during arousal from sleep that is, desynchronization of the large amplitude, slow waves associated with sleep and their replacement by small amplitude, diffuse, fast activity Lesions in the upper part of the brain stem, confined to the medial part where the reticular formation lies, blocked these responses, whilst similar lesions in chronic animal preparations caused chronic loss of wakefulness. Since the findings of Moruzzi & Magoun many workers have investigated this system and we know now a great deal more about its anatomy and physiology

The present concept of the reticular activating system is that it exerts a tonic ascending facilitatory influence on the higher centres of the brain, particularly on the cerebral cortex, and when this influence is suppressed or removed, sleep or coma results. The reticular activating system is itself influenced by incoming sensory impulses travelling along the afferent pathways and it is thought that this takes place at the brain stem level, via collaterals from these pathways into the reticular formation (Pl. 1) Thus, sensory messages can affect the level of tonic activity in the reticular formation and through its ascending influence, the level of cortical activation and state of wakefulness In this way a sensory stimulus may reach the cerebral cortex through two pathways. First, it travels along the specific sensory pathway from the periphery and eventually reaches the appropriate sensory receiving area of the cortex. At the same time, when it reaches the brain stem, it also enters the reticular formation where, if its intensity is high enough, it raises the level of tonic activity sufficiently to cause widespread cortical activation and arousal. In this system, however the stimulus loses its identity with the original sensation and we find that the activation produced by an auditory stimulus is exactly the same as that produced by tactile or visual stimuli.

In considering the actions of drugs in relation to the reticular activating system, those drugs which are known to affect states of wakefulness and consciousness, such as central excitants like amphetamine, and depressants such as the barbiturates, might well be expected to act on this system. In fact, there is now good evidence that these particular drugs produce their effects by an action on the reticular formation, amphetamine by an excitant action (Bradley & Elkes, 1957) and barbiturates by a depressant action (French, Verzeano & Magoun, 1953).

Returning now to the tranquillizers, we think that their action on the arousal system is related more specifically to the influence of afferent

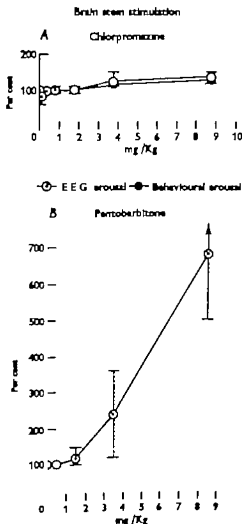
impulses on the reticular formation. The evidence for this suggestion has been obtained from experiments in which we have examined the effects of drugs on thresholds for arousal produced by (*a*) direct stimulation of the reticular formation and (*b*) sensory stimulation (Bradley & Key 1958). Direct stimulation of the reticular formation was achieved by means of a technique similar to that used by Moruzzi & Magoun, a coaxial stimulating electrode being inserted stereotactically and the electrical activity of the cortex recorded whilst stimuli of increasing intensity were applied. In this way the threshold for arousal responses could be determined, both before and after the administration of drugs, and graphs plotted of the threshold against the dose of the drug Such graphs have been made for a number of different barbiturates and it is interesting to compare these with chlorpromazine (Text fig 1)

All the barbiturates which we have examined caused a rise in the threshold for arousal produced by direct stimulation of the reticular formation and at dose levels of 8–10 mg /Kg arousal responses were completely blocked (Text fig 1*B*) At the same time arousal produced by external sensory stimuli was blocked Chlorpromazine, on the other hand, caused the threshold for arousal produced by stimulation of the reticular formation to rise only slightly even when comparatively large doses (8–9 mg /Kg) were injected (Text-fig 1 *A*) but arousal produced by sensory stimulation was blocked by much lower dose levels of this drug (2–3 mg /Kg)

It has been found that barbiturates block the conduction of impulses in the brain stem reticular formation (French *et al* 1953 Hance, 1958) and this could account for the blocking of arousal responses by these drugs, both for sensory and direct stimulation. Chlorpromazine does not affect the conduction of impulses in the brain stem (Killam & Killam, 1957 Hance 1958) and as it had little effect on arousal produced by stimulation of the reticular formation, it appears that this drug has little depressant action on this system as compared with that possessed by the barbiturates. Thus, the blocking of arousal to sensory stimuli would seem to be a more specific action in the case of this drug and this led us to suggest that chlorpromazine has a depressant action which is associated with afferent influences on the reticular formation and that its site of action may be closely related to the afferent collaterals entering the brain stem reticular formation (Bradley & Key 1958)

Further support for this hypothesis comes from experiments in which the activity of single neurones in the brain stem has been recorded with micro-electrodes. Many of these neurones show convergence responses, that is, they respond to more than one mode of sensory stimulation. After chlorpromazine had been injected all such neurones showed greatly

diminished responses when the same stimulus was applied (Bradley 1958). There are, on the other hand, findings which do not fit in well with this hypothesis and these have demonstrated that certain electrical responses



Text-figure Graphs showing the effects of (*A*) chlorpromazine, and (*B*) pentobarbitone on the thresholds for arousal produced by direct stimulation of the reticular formation. The mean values of the percentage change in threshold have been plotted against dose. (From Bradley P. B. & Key B. J. (1958). *Electroenceph. Clin. Neurophysiol.* [illegible], [illegible].)

recorded from the reticular formation are enhanced by the administration of chlorpromazine and not reduced (Killam & Killam, 1958). However it is difficult at the present time to suggest the possible functional significance of these responses and their relation to the activity of single neurones.

Other workers have shown that although the amplitude of these slow potentials may be augmented by chlorpromazine, their recovery cycle is retarded (De Maar Martin & Unna, 1958).

Our hypothesis for the mode of action of chlorpromazine is a very tentative one and further investigations will have to be carried out with this drug We must not, at this stage, suggest that it is this particular action of the drug which is responsible for its tranquillizing action in man but it is interesting to speculate on this possibility Certainly if we accept that the ascending influence of the arousal system determines our state of wakefulness and possibly affects our reactions to sensory stimuli, then any interference with the influence of such stimuli on this system might well upset this relationship so that our reactions to the stimuli are altered although they may still be reaching the cerebral cortex.

Finally it must be remembered that the tranquillizers are a far from homogeneous group and differ from one another in almost all their properties. Thus, the experimental findings and tentative hypothesis which I have outlined apply to only chlorpromazine, and other tranquillizers which we have studied appear to have different properties. This is certainly true of reserpine which produced a dissociation between EEG activity and EEG behaviour Others such as azacyclonol and meprobamate appear to have no effect on the arousal system whilst hydroxyzine is in some ways similar to chlorpromazine (Bradley & Key 1958).

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EXPLANATION OF PLATE

PLATE

Lateral view of the monkey brain, showing the ascending reticular activating system in the brain stem, receiving collaterals from direct afferent paths and projecting to all areas of the cerebral cortex (From Magoun, H. W (954). I *Brain Mechanisms and Consciousness* Oxford Blackwell).

DISCUSSION

Chairman DR H R. VICKERS (Oxford)

VICKERS. Do corticosteroids influence the response to other drugs? I think emotion alters tissue reactions, and I wonder whether it acts in some way through hormone stimulation, perhaps through the corticosteroid mechanism?

DR G B DOWLING (London). We take blushing for granted I wonder if Professor Paton would enlarge on the subject and help us to understand why people differ so much, why some people can vasodilate or blush, and why some do not. We take the mechanism for granted but I do not think we have ever understood it.

PROFESSOR W D M PATON (London). The chairman and Dr Dowling have raised the question of autonomic behaviour varying I do not know whether there is any good evidence that cortical hormones can influence this although it has been gone into as regards peripheral effects on autonomic behaviour there does not seem to be very much to go for so far as ordinary fluctuations in cortical activity are concerned. Central effects of cortical hormones might be different. The theory which appeals to me most as to why people vary autonomically is that the autonomic system transmits the visceral expression of our personalities this means that, in so far as our personalities differ our autonomic behaviour will differ too. Of course a ganglion-blocking agent can only show itself if autonomic activity is there to be blocked. It is the same with curarization if you have a completely relaxed individual on artificial respiration, you cannot tell by inspection whether he has curare in him or not. Similarly with autonomic block if an autonomic pathway is silent you do not know if it is blocked or not. Hence, if autonomic activity varies, the pattern of ganglion block varies. There is another factor too the faster and longer a given autonomic pathway is excited the bigger in proportion is the block produced by a ganglion-blocking agent—again rather like the curarized patient or the myasthenic man. Whereas brief single shocks may elicit a response the synapse becomes paralysed with sustained excitation. This would be another mechanism which picks out those autonomic pathways which are most active in the body those pathways which are most active should be affected first. This explains, for instance the occasional dramatic fall in blood pressure in hypotensives, with rather small doses of hexamethonium One can suppose that in such patients the vasomotor ganglia were being activated at a very rapid rate with a correspondingly enhanced sensitivity

I introduced blushing into the discussion only because, whilst preparing my paper it occurred to me that this is one of the commonest causes of vasodilatation. I have not found a fully satisfactory account of it. It is remarkable that one has to turn to Darwin for an account of blushing Lewis's book on the skin refers largely to him and to a contemporary The great difficulty for investigation, of course, is to produce a standard blush. I have tried to make my laboratory assistant blush but he never will! Sir Thomas Lewis, I think, has explained it as mediated by reduction in sympathetic tone. But I am not quite convinced, partly because a patient who is vasodilated does not look like someone who is blushing I hoped that somebody here might be able to tell me whether a sympathectomized man can blush. Is there a pathological blush which presents to dermatologists and which has some other accompanying features? For instance, does the occasional individual who blushes really extensively possibly down to the chest, feel faint at the same time? Or does anything else happen when you blush—stuffiness of the nose or increase in secretions? If blushing is due to something other than release of sympathetic vasoconstriction, it might be rather interesting

VICKERS. Could the difference in action between the hydrogenated and unhydrogenated ergot compounds be explained by the fact that the hydrogenated compounds are able to cross the blood-brain barrier and so act centrally whilst the unhydrogenated can only act peripherally?

PATON That is an interesting suggestion and chemically there might be something in it. However other central actions have been described for the unhydrogenated alkaloids. It looks as though both the unhydrogenated and hydrogenated compounds can penetrate the blood-brain barrier

DR N R. ROWELL (Leeds). I am interested to hear that Professor Paton finds that mecamylamine has an action of about 24 hr There is no doubt that the duration of action of ganglion-blocking drugs in experimental work differs from the duration in practice.

In my experience mecamylamine has to be given two to three times a day to have an adequate hypotensive effect. I agree with the variability of absorption and I have seen several cases of paralytic ileus due to this drug Constipation seems to be an almost invariable side effect, although prostigmine is sometimes effective in counteracting this. Pilocarpine is useful for dryness of the mouth, eserine drops for the prevention of glaucoma and the prescription of new spectacles allow for the pupillary changes. Impotence is a further side effect. This can be helped by omitting the evening dose.

I would like to ask what is the mechanism of depression caused by reserpine. Is there a centre for depression? I have seen Parkinsonism due

to reserpine develop in five days. This disappeared within a week of stopping the drug

Lastly I would like to ask Professor Paton what he thinks of the effect of alcohol as a vasodilator Professor Rob considers it is much more effective than drugs.

PATON Dr Rowell corrected my remarks on mecamylamine, quite rightly as far as clinical practice goes. There is a point which arises here between the laboratory and clinical work. If I say a drug works for a given length of time what one means is that in the laboratory one can detect signs, however small of the presence of that drug over this period. But the laboratory tests are more sensitive than clinical tests. Clinically one is interested in the period of time over which a significant effect is obtained. So that I would not in the least disagree with his correction, and I am glad he made it.

Dr Rowell also asked about reserpine and why it produces depression. This is one of the 1000 dollar questions perhaps Dr Bradley might be able to comment on it.

Then alcohol as a vasodilator I think there is something in it. All the anaesthetics are in fact vasodilators, peripherally and alcohol of course is one of the classical anaesthetics. One imagines they act by depressing the excitability of a contractile tissue, so that it spends more time in the relaxed state. It seems quite clear that alcohol can be quite useful in this sort of way the main difficulty being its other actions.

DR P B BRADLEY (Birmingham) The answer to the question about reserpine and depressions is that we do not know why these depressions should occur any more than we know why some of these drugs cause symptoms of Parkinsonism. I am sure we should not think in terms of a centre for depression or for any of these functions, and I hope that my diagrams did not suggest that we think of the arousal system as a centre. The reticular formation is a very diffuse structure which seems to be concerned in a wide variety of functions and this may be the reason why drugs like chlorpromazine have such a variety of effects.

REFERENCE

LEWIS T (1927) *The Blood Vessels of the Human Skin and their Responses* London Shaw and Sons, Ltd.

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